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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|  |    |   |
|--|----|---|
| (51) International Patent Classification <sup>7</sup> :<br>C12N 15/12, 15/57, 15/67, 15/85, 9/64,<br>C07K 14/72, C12Q 1/68, A61K 48/00 | A1 | (11) International Publication Number: <b>WO 00/49147</b><br>(43) International Publication Date: 24 August 2000 (24.08.00) |
|--|----|---|

(21) International Application Number: PCT/EP00/01368

(22) International Filing Date: 18 February 2000 (18.02.00)

## (30) Priority Data:

|              |                             |    |
|--------------|-----------------------------|----|
| 199 07 099.7 | 19 February 1999 (19.02.99) | DE |
| 60/120,848   | 19 February 1999 (19.02.99) | US |

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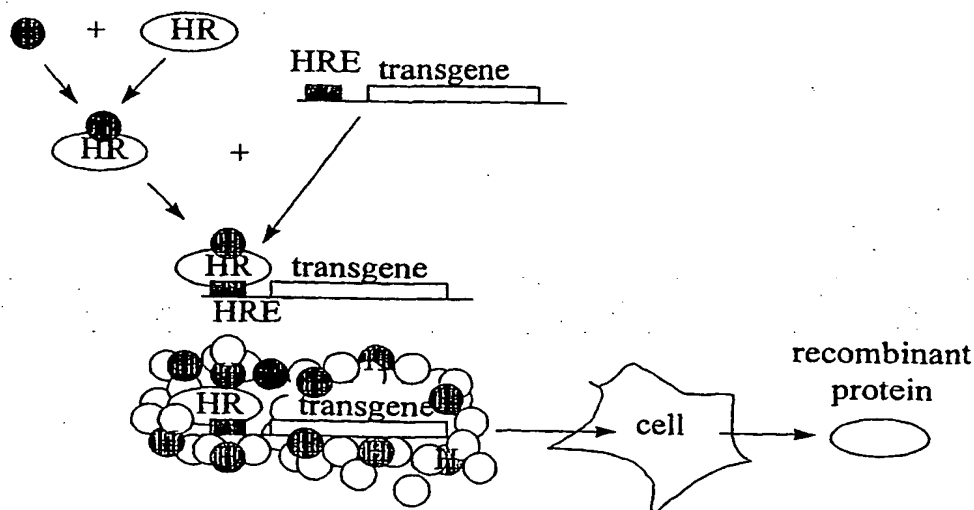
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

## Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HORMONE-HORMONE RECEPTOR COMPLEXES AND NUCLEIC ACID CONSTRUCTS AND THEIR USE IN GENE THERAPY



## (57) Abstract

The invention relates to the use of a nucleic acid construct comprising at least one hormone responsive element and a transgene for preparing an agent for gene transfer. It further relates to particular nucleic acid constructs comprising at least one hormone responsive element and a transgene, wherein one of said at least one hormone responsive elements is not functionally linked to the transgene, vectors comprising such nucleic acid constructs and compositions of matter comprising such nucleic acid constructs wherein the hormone responsive elements of the constructs are coupled to a hormone-hormone receptor complex. The nucleic acid constructs, plasmids, and compositions of matter of the invention have applications in gene therapy, particularly in the treatment of human blood clotting disorders, such as hemophilia. They may also be used to up- or down-regulate target genes and for the delivery of vaccines.

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# **Hormone –Hormone Receptor Complexes and Nucleic Acid Constructs and Their Use in Gene Therapy**

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## **Background of the Invention**

### **1. Object of the Invention**

The invention relates to the use of a nucleic acid construct comprising at least one hormone responsive element and a transgene for preparing an agent for gene transfer. It further relates to particular nucleic acid constructs comprising at least one hormone responsive element and a transgene, wherein one of said at least one hormone responsive elements is not functionally linked to the transgene, vectors comprising such nucleic acid constructs and compositions of matter comprising such nucleic acid constructs wherein the hormone responsive elements of the constructs are coupled to a hormone-hormone receptor complex. The nucleic acid constructs, plasmids, and compositions of matter of the invention have applications in gene therapy, particularly in the treatment of human blood clotting disorders, such as hemophilia. They may also be used to up- or down-regulate target genes and for the delivery of vaccines.

### **2. Summary of the Related Art**

Gene therapy is a method that holds great promise for many diseases and disorders. In general, it involves the transfer of recombinant genes or transgenes into somatic cells to replace proteins with a genetic defect or to interfere with the pathological process of

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an illness. In principle, gene therapy is a simple method. In practice, many disadvantages must still be overcome.

Research in gene therapy has concentrated on ways to most effectively incorporate DNA into cells of a patient. Viral vectors are currently the widely used vehicles in clinical gene therapy approaches. In terms of efficacy in gene expression, the viral delivery systems have major advantages over techniques using DNA-lipid formulations as delivery vehicles or over mechanical methods, such as the gene gun. Although there are a variety of viral systems tested for gene therapeutical strategies, retroviral vectors and adenoviral vectors are presently the most widely used vehicles (Salmons, B. and Gunzburg, W. H., *Hum. Gene Ther.*, Vol. 4, 129, 1993; Kasahara, N. A., et al., *Science*, Vol. 266, 1373, 1994; Ali, M., et al., *Gene Ther.*, Vol. 1, 367, 1994. ). Still, these systems have major disadvantages, such as potential viral contamination. Other safety concerns continue to hamper the development of clinical application of gene therapy using these viral systems. For example, recombinant retroviruses have the disadvantage of random chromosomal integration, which may lead to activation of oncogenes or inactivation of tumor-suppressor genes. Also, repetitive use of recombinant adenoviruses has caused severe immunological problems (Elkon, K. B. et al., *Proc. Natl. Acad. Sci. USA*, Vol. 94, 9814, 1997). The humoral response resulted in the production of antibodies to adenovirus proteins preventing subsequent infection. Immunosuppressive drugs may ameliorate these effects, but they place an additional burden on the patient (Dai, Y., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 92, 1401, 1995).

Yet another viral delivery system involves adenoassociated virus (AAV). The AAV requires coinfection with an unrelated helper virus. Although such recombinant AAV virions have proven useful for introducing several small gene sequences into host cells, gene

delivery systems based on those particles are limited by the relative small size of AAV particles. This feature greatly reduces the range of appropriate gene protocols. Moreover, the need to also use a helper virus adds a complicating factor to this delivery system (Muzyczka, N.,  
5 Curr. Top. Microbiol. Immunol., Vol. 158, 97, 1992).

Though safer, non-viral gene therapy approaches are also unsatisfactory. Problems with inefficient gene delivery or poor sustained expression are major drawbacks. Yet the methods available such as the direct injection of DNA into cellular compartments, or the  
10 application of mixtures of DNA with cationic lipids or polylysine allowing the transgene to cross the cell membrane more easily, have not overcome these hurdles (Felgner, P., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 84, 7413, 1987; Behr, J.-P., *Bioconjugate Chemistry*, Vol. 5, 382, 1994).

15 Introduction of naked DNA (polynucleotide) sequences (including antisense DNA) into vertebrates, is reported to be achieved by injection into tissues such as muscle, brain or skin or by introduction into the blood circulation (Wolff, J. A., et al., *Science*, Vol. 247, 1990; Lin, H., et al., *Circulation*, Vol. 82, 2217, 1990; Schwartz, B., et al.,  
20 *Gene Ther.*, Vol. 3, 405, 1996). Also, a direct gene transfer into mammals has been reported for formulations of DNA encapsulated in liposomes and DNA entrapped in proteoliposomes containing receptor proteins. Although injected naked DNA leads to transgene expression, the efficiency is by far not comparable to viral-based DNA delivery  
25 systems. A limitation of the method of naked DNA injection is the fact that transgene expression is dose-dependent. The gene expression is saturable, and an increase in the amount of DNA injected leads to decreased protein production per plasmid. Thus, protein expression can dramatically decrease, if the amount of DNA injected is above a  
30 certain threshold.

Among the genetic disorders that the skilled artisan has sought to overcome using these prior art methods are those relating to blood clotting disorders, and in particular, hemophilia (Lozier, J. N. and Brinkhous, K. M., *JAMA*, Vol.271, 1994; Hoebe, R. C., *Biologicals*, Vol. 23, 27, 1995). For example, hemophilia A and B are X-linked, recessive bleeding disorders caused by deficiencies of clotting factors VIII and IX, respectively (Sadler, J. E. et al., in: *The Molecular Basis of Blood Diseases*, 575, 1987). The incidence of hemophilia is about 1 in 5,000 male births. Hemophiliacs suffer from excessive bleeding due to the lack of clotting at the site of wounds. The inability to clot properly causes damage to joints and internal tissues as well as posing risks to the proper treatment of cuts.

Treatment of hemophilia A is possible by the administration of the blood clotting factor VIII. Until recently, factor VIII preparations had to be prepared by concentrating blood from donors, posing the risk of contamination by infectious agents, such as HIV and hepatitis. The gene for factor VIII has been cloned (e.g., Vehar et al., *Nature* Vol. 312, 337 1984) allowing for the production of a recombinant product. Although recombinant methods provide factor VIII of higher purity than blood concentrates, the exogenous supply of factor VIII to a patient still requires repeated doses throughout the lifetime of the patient, an inconvenient and expensive solution.

Other forms of hemophilia include hemophilia B, caused by a defect in the gene coding for Factor IX. The gene therapy systems described above have been attempted for the treatment of hemophilia A and B with factors VIII and IX, respectively. (See e.g., WO 94/29471). However, these systems have the disadvantages already discussed above.

On the other hand, the classical model of the action of hormones is based on the concept of binding interaction of the hormone to an

intracellular receptor, located in the cytoplasm or the nucleus (Evans, R., *Science*, Vol. 240, 889, 1988). These intracellular receptors remain latent until exposed to their target hormone. When so exposed, the hormone receptor changes its conformation after the hormone is bound and translocates in the activated form into the cell nucleus where it binds as a dimer to hormone responsive elements in the promoter region of hormone-regulated genes (Beato, M., *Cell*, Vol. 56, 335, 1989; O'Malley, B., et al., *Biol. Reprod.*, Vol. 46, 163, 1992). The hormone responsive elements are enhancer elements usually located in the 5' flanking region of the specific hormone-induced gene, i.e., are functionally linked to the specific hormone induced gene. DNA constructs comprising a hormone responsive element and a nucleic acid sequence encoding a protein of interest are disclosed in U.S. Pat. Nos. 5,688,677 and 5,580,722 and are taught to be suitable for expression of the protein of interest.

An example of such intracellular receptors is the steroid receptor. Steroid receptors belong to a superfamily of ligand-dependent transcription factors characterized by a unique molecular structure. The centrally located highly conserved DNA-binding domain defines this superfamily. The second important and relatively invariant region is the COOH-terminal ligand-binding domain. An example of such a receptor is the progesterone receptor mediated by the steroid progesterone. At the progesterone receptor, progesterone acts as a natural agonist whereas it displays potent antimineralocorticoid properties both at the molecular and the systemic level. Besides classical effects on the uterus, antiepileptic, anxiolytic, hypnotic and anesthetic properties have been attributed to progesterone according to numerous studies.

Methods have been proposed for the use of mutant hormone receptors, including mutant steroid receptors for gene therapy. For



example, such methods are disclosed in WO 93/23431, WO 98/18925, WO 96/40911. Moreover, WO 98/33903 discloses a genetic construct comprising a steroid responsive element from a tissue specific gene, a coding sequence, and an SV40 enhancer.

5

### **Brief Description of the Invention**

The object of the present invention is to overcome the disadvantages of the previous gene therapy delivery systems. It was found that a hormone-hormone receptor complex possesses the ability to drag a nucleic acid construct having one or more hormone responsive element(s) through the cell membrane into a cell. It was also found that if the construct comprises further functional sequences besides the hormone responsive elements (hereinafter "transgenes"), the functional sequences exert their function. The hormone responsive element may also enhance the expression of the transgene. Moreover, it was found that steroid hormones are very effective mediators for the transfer of nucleic acid constructs through the cell membranes into a cell. The present invention thus provides

(1) the use of a nucleic acid construct comprising at least one hormone responsive element (hereinafter referred to as "HRE") and a transgene for preparing an agent for gene transfer (said at least one HRE being functionally linked to the transgene or not);

(2) a preferred embodiment of (1) above, wherein the agent further comprises a hormone-hormone receptor complex;

(3) a nucleic acid construct comprising at least one HRE and a transgene, wherein one of said at least one HREs is not functionally linked to the transgene;

(4) a vector comprising the nucleic acid construct of (3) above;

(5) a transformed cell or transgenic organism comprising the nucleic acid construct as defined in (3) above or the vector as defined in (4) above;

(6) a composition of matter comprising a nucleic acid construct comprising at least one HRE and a transgene as defined in (3) above and/or a vector as defined in (4) above, said at least one HRE being coupled to a hormone-hormone receptor complex;

(7) a preferred embodiment of (6) above, wherein the transgene is a gene encoding a blood clotting factor;

(8) a preferred embodiment of (7) above, wherein the blood clotting factor is factor IX;

(9) a preferred embodiment of (7) above, wherein the blood clotting factor is factor VIII;

(10) a pharmaceutical composition comprising the nucleic acid construct as defined in (3) above and/or the composition of matter as defined in (6) to (9) above;

(11) a method for preparing the composition of matter as defined in (6) above, which method comprises admixing the nucleic acid construct with the hormone receptor and the hormone;

(12) a method for gene transfer which comprises administering the agent as defined in (1) and (2) or the composition of matter as defined in (6) to (9) above to an organism or to a cellular system;

(13) a method for delivering into an organism or into a cellular system a nucleic acid encoding a transgene to be expressed in the cells of the organism or the cells of the cellular system, which method comprises administering an agent as defined in (1) above or composition of matter as defined in (6) to (9) above to the organism or to the cellular system so that the hormone in the composition interacts with the cell membrane and therewith enhances diffusion

and transport of the nucleic acid that is coupled to the hormone-hormone receptor complex across the membrane and into the cell;

(14) a method of treating blood clotting disorders comprising administering a therapeutically effective amount of the composition of matter as defined in (7) above to an organism or to a cellular system;

(15) a method of treating hemophilia B, comprising administering a therapeutically effective amount of the composition of matter as defined in (8) above to an organism or to a cellular system;

(16) method of treating hemophilia A, comprising administering a therapeutically effective amount of the composition of matter as defined in (9) above to an organism or to a cellular system;

(17) use of a steroid hormone for preparing an agent for gene transfer; and

(18) a method for gene transfer which comprises administering a nucleic acid construct to an organism or to a cellular system, wherein the nucleic acid construct contains a transgene and is encapsulated in a steroid hormone.

In a preferred embodiment of (1) to (16) above the hormone responsive element is a steroid responsive element (SRE), most preferably a progesterone responsive element (PRE). In embodiments (2) and (6) to (16) the receptor preferably is a steroid receptor, most preferably, a progesterone receptor. Similarly, the hormone is preferably a steroid, most preferably, progesterone.

The present invention thus provides a delivery system for gene therapy that should overcome the prior art disadvantages. The presence of the hormone responsive element on the nucleic acid carrying a transgene encourages the binding of a hormone-hormone receptor complex. Thus, the present invention uses the activated hormone receptor as a link (or binding compound) between the

nucleic acid carrying the transgene and the hormone known to interact with the cell membrane. The general known biological activity mediated by the HREs is not the primary effect utilized in the present invention, but might be an additional effect when regulation of the transgene is desired. The general principle is depicted in Figure 1. The hormone responsive element is preferably present as a nucleic acid dimer sequence or nucleic acid multimer sequence. Even in an inverse orientation, the hormone responsive element will exert its proper function. The hormone-hormone receptor complex contains a hormone receptor that becomes activated after binding of its specific hormone. The hormone receptor in the activated state is able to recognize and bind to its specific hormone responsive element, which in the present invention is present within the nucleic acid comprising the desired transgene, e.g., a human blood-clotting factor.

Vaccination is another aspect of the embodiment (12) defined above. Introducing a nucleic acid construct or composition of matter of the invention comprising a gene for an antigen or containing a viral sequence into a cell (DNA vaccines) using the method mentioned above may also provide a way to stimulate the cellular immune response.

### **Brief Description of the Drawings**

Figure 1 shows the concept of gene transfer of the present invention (with HRE = hormone responsive element, HR = hormone receptor, H = hormone, blank circles = lipophilic matrix).

Figure 2 is a diagram of the vector pTGFG1.

Figure 3 is a diagram of the vector pTGFG5.

Figure 4 is a diagram of the vector pTGFG20.

Figure 5 is a diagram of the vector pTGFG33.

Figure 6 is a diagram of the vector pTGFG36.

Figure 7 is a diagram of the vector pTGFG53.

Figure 8 is a diagram of the vector pTGFG64.

Figure 9 is the DNA sequence of vector pTGFG36 (SEQ ID NO: 1).

5 Figure 10 shows the protein sequence of factor IX encoded by vector pTGFG36 (SEQ ID NO: 2).

Figure 11 shows a GFP concentration curve for cell homogenates after transfection with pTGFG5 and pTGFG20, respectively.

Figure 12 shows corresponding light (a and c) and fluorescent (b and  
10 d) micrographs of HeLa cells transfected with pTGFG5 (a and b) and pTGFG20 (c and d), respectively.

Figure 13 shows the amount of GFP expressed by utilizing the favoured vectors of the invention in a transfection experiment. Relative fluorescence units from mock and background can be clearly  
15 separated.

Figure 14 shows the additive effect of human clotting factor IX on clotting activity of mouse blood.

Figure 15: hPR (A-form) was expressed in insect cells and purified by cobalt<sup>2+</sup> affinity chromatography as described in Example 5. The final  
20 preparation (85µg protein) was separated on a denaturing 7,5% SDS-polyacrylamid gel, followed by staining with coomassie® R250 (lane A) or western blotting with hPR-specific staining (lane C).

Lane B: Molecular mass standard. Arrows indicate the two highly enriched protein species (94 and 74 kDa) accessible to  
25 immunodetection.

Figure 16: Domain structure of hPR-B (numbers on the top of the bar represent amino acid positions within the polypeptide sequence).

Figure 17 shows the mean values of the difference in the clotting time of Example 9.

30 Figure 18 shows the clotting time detected in Example 9.

Figure 19 shows the activity of human progesterone receptor as determined in Example 8.

Figure 20: shows the amino acid sequence of the hPR B-Form. The start methionine 165 of the hPR A-Form is underlined (SEQ ID NO: 18).

Figure 21 shows the nucleic acid sequence of the mRNA coding for hPR. The reading frame for the hPR B-form starts at position 176, the reading frame for the hPR A-Form at position 668. The respective start codons ATG are underlined (SEQ ID NO: 19). The sequences of Figures 20 and 21 are taken from Genbank, accession number AF016381.

## Detailed Description of the Invention

### 1. Definitions

"Nucleic acid" means DNA, cDNA, mRNA, tRNA, rRNA. The nucleic acid may be linear or circular, double-stranded or single-stranded.

"Nucleic acid construct" refers to a composite of nucleic acid elements in relation to one another. The nucleic acid elements of the construct may be incorporated into a vector in such an orientation that a desired gene may be transcribed, and if desired, a desired protein may be expressed.

"Transgene" refers to a functional nucleic acid sequence which is transcriptionally active (with or without regulatory sequences).

"Gene transfer" includes "gene therapy".

"Hormone responsive element" (HRE) refers to regions of nucleic acids, and in particular, DNA, which regulate transcription of genes in response to hormone activation. HREs are typically about 10-40 nucleotides in length, and more usually, about 13-20 nucleotides in

length. As explained above, HREs become activated when a hormone binds to its corresponding intracellular receptor causing a conformational change, so that the receptor has increased affinity for the HRE and binds to it. The HRE, in turn, stimulates transcription. A "steroid responsive element" (SRE) is an HRE that regulates transcription of genes in response to steroid activation. A "progesterone responsive element" (PRE) is an HRE/SRE that regulates transcription of genes in response to progesterone activation.

A "hormone receptor" refers to a receptor which binds to and is activated by a hormone. A "steroid receptor" refers to a receptor which binds to and is activated by a steroid hormone. A "progesterone receptor" is a receptor which binds to or is activated by the steroid hormone progesterone.

"Functionally linked" refers to configurations of the nucleic acid construct, where the HRE (or SRE/or PRE) is located within the construct so that it can stimulate transcription of the transgene. "Not functionally linked" refers to configurations where the HRE is so remotely located from the transgene that it cannot stimulate its transcription.

"Gene" refers to DNA sequence encoding a polypeptide, optionally including leader and trailer sequences and introns and exons.

"Vector" refers to any genetic construct, such as a plasmid, phage, cosmid, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. The term includes cloning and expression vehicles.

"Promoter" refers to a region of regulatory DNA sequences for the control of transcription of a gene to which RNA polymerase binds.

The promoter forms an initiation complex with RNA polymerase to initiate and drive transcription activity. "Enhancers" may activate the complex or "silencers" may inhibit the complex. A "tissue-specific promoter" is a promoter found in the DNA of tissue for transcription of genes expressed in this specific tissue.

"Organism" refers to a multicellular living entity including vertebrates such as mammals (especially humans, cattle, rodents, dogs) and invertebrates.

"Cellular system" includes cell cultures, e.g., primary cell cultures (especially those suitable for reimplantation), stem cells, blood cells, tissue samples and whole organs and immortalized cell cultures.

"Therapeutically effective dose" of the products of the invention refers to a dose effective for treatment or prophylaxis, for example, a dose that yields effective treatment or reduction of the symptoms of hemophilia. It is also a dose that measurably activates expression of a target gene as determined by measurements of target protein levels, or a dose that is predictable to be effective for treatment or prophylaxis by extrapolating from *in vitro* or *in vivo* data. The determination of a therapeutically effective dose is within the purview of one skilled in the art.

"Encodes" or "encoding" refers to a property of the nucleic acid sequence of being transcribed (in case of DNA) or translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences.

For the purposes of this application, "express", "expressing" or "expression" shall refer to transcription and translation of a gene encoding a protein.



## 2. Detailed Description and Examples

As stated above, an object of the present invention is to provide a new and improved delivery system for gene therapy. The invention thus provides nucleic acid constructs comprising at least one HRE and a transgene wherein one of said at least one HREs is not functionally linked to the transgene, and compositions of matter comprising such nucleic acid construct wherein said at least one HRE is coupled to a hormone-hormone receptor complex (embodiments (3) and (6) defined above). A preferred embodiment of the nucleic acid construct and of the composition of matter of the invention is one where the hormone responsive element is a steroid responsive element (SRE), and the receptor is a steroid receptor. Most preferably, the hormone responsive element is a progesterone responsive element (PRE), and the receptor is a progesterone receptor.

Potential HREs for use in the present invention have been previously described. For example, GREs (Scheidereit, C., et al., *Nature*, Vol. 304, 749, 1983; von der Ahe, D., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 83, 2817, 1986), EREs or PREs (Chambon, P., et al., *Rec. Prog. Horm. Res.*, Vol., 40, 1, 1984; Klock, G., et al., *Nature*, Vol. 329, 734, 1987). As already stated above, the most preferred HRE for the invention is a PRE. Specifically, the preferred PRE is described in Example 1, i.e., is the double stranded DNA sequence comprised of SEQ ID NOs: 3 and 4. The nucleic acid for use in the invention comprises at least one hormone responsive element. Preferred is a nucleic acid comprising more than one HRE. For example, the nucleic acid may comprise three to ten, preferably three to five HREs. The most preferred embodiment is a nucleic acid comprising three to five PREs.

Potential hormone receptors for use in the present invention are, for example, estrogen receptors, mineralocorticoid receptors,

glucocorticoid receptors, retinoic acid receptors, androgen, calcitriol, thyroid hormone or progesterone receptors and orphan receptors. Such receptors have been previously described. (Green, S., et al., *Nature*, Vol. 320, 134, 1986; Green, G. L., et al., *Science*, Vol. 231, 1150, 1986; Arriza, J. L., et al., *Science*, Vol. 237, 268, 1987; Hollenberg, S. M., et al., *Nature*, Vol. 318, 635, 1985; Petkovitch, M., et al., *Nature*, Vol. 330, 444, 1987; Giguere, V., et al., *Nature*, Vol. 330, 624, 1987; Tilley, W., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 86, 327, 1989; Baker, A. R., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 85, 3294, 1988; Weinberger, C., et al., *Nature*, Vol. 324, 641, 1986; Sap, J., et al., *Nature*, Vol. 324, 635, 1986; Misrahi, M., et al., *Biochem. Biophys. Res. Commun.*, Vol. 143, 740, 1987; Kastner, P., et al., *Cell*, Vol. 83, 859, 1995). These receptors may be from human or other mammalian sources, although human is preferred. Nucleotide and/or amino acid sequences of human steroid receptors are available in the GenBank: mineralocorticoid receptor: M16801; glucocorticoid receptor  $\alpha$ : M10901; glucocorticoid receptor  $\alpha_2$ : U01351; glucocorticoid receptor  $\beta$ : M11050; retinoic acid receptor  $\alpha$ : AF088888 (exon 1), AF088889 (exon 2), AF088890 (exon 3), AF088891 (exon 4), AF088892 (exon 5 and 6), AF088893 (exon 7), AF088894 (exon 8), AF088895 (exon 9 and complete cDNA); retinoic acid receptor  $\gamma$ : M24857; androgen receptor: M27423 (exon 1), M27424 (exon 2), M27425 (exon 3), M27436 (exon 4), M27427 (exon 5), M27428 (exon 6), M27429 (exon 7), M27430 (exon 8); thyroid hormone receptor  $\alpha_1$ : M24748, thyroid hormone receptor  $\alpha_2$ : J03239; progesterone receptor: AF016381; somatotropin receptor: J00148; vitamin D receptor (calcitriol receptor): J03258.

The skilled person will understand that expression of the receptor proteins can be achieved by standard methods, e.g. via PCR-cloning of the known cDNAs from cDNA libraries and overexpression of

the corresponding proteins in suitable expression vectors, such as, for example, the vectors of the present invention, in suitable host cells, e.g., COS cells. Accordingly, subsequent purification of the cytosolic fraction can be achieved by routine methods such as affinity chromatography purification. For this purpose, various suitable antibodies against the desired receptor are commercially available. For example, polyclonal antibodies against the mouse progesterone receptor that have a sufficiently high cross-reactivity for the human protein are available from Dianova (Hamburg, Germany). Likewise, further purification can be achieved by standard methods, e.g., chromatographical methods such as ion-exchange chromatography and/or FPLC.

The most preferred receptor is the progesterone receptor. Preferably, the receptor is a human progesterone receptor. Such a human progesterone receptor (from T47D human breast cancer cells) is disclosed in US Patent No. 4,742,000, and cells expressing this receptor have been deposited (ATCC deposit number HTB, 133). As already described above, it would be routine to purify such a receptor from the cytosol using receptor specific antibodies. In addition, US Patent No. 4,742,000 discloses a method for purification of the human progesterone receptor using a specific steroid affinity resin (cf. Grandics et al., Endocrinology, Vol. 110, 1088, 1982).

Briefly, the cytosolic fraction of the T47D cells is passed over Sterogel, a commercial preparation of deoxycorticosterone coupled to Sepharose® 2B that selectively binds the progesterone receptor. After washing with loading buffer, the bound receptor is eluted with a buffer containing progesterone. The eluted steroid-receptor complex is then chromatographed on DEAE-Biogel and eluted stepwise with a buffer containing 0.2M NaCl. Subsequently, the bound progesterone can be

readily exchanged. As described above, further purification can be achieved by routine methods well-known to the skilled person.

An alternative method is disclosed in Example 5.

The structure of the hPR polypeptide is depicted in Fig. 16. The hPR polypeptide is composed of distinct structural domains. Naturally the human progesterone receptor (hPR) is expressed as two different sized proteins termed hPR-B (120 kDa) and hPR-A (94 kDa). HPR-A is a truncated but otherwise identical form of hPR-B, that is missing 165 the N-terminal amino acids (see Fig. 20, SEQ ID NO: 18). Both forms seems to be indistinguishable regarding their progesterone or DNA binding properties. In human cells the A and B forms of hPR are produced from the same gene by alternate initiation of translation at two different AUG start sites within the same RNA transcript. As it was reported earlier hPR-A and B can be expressed in *Spodoptera frugiperda* (Sf9) cells as biological fully active polypeptides (Christensen *et al.*, Mol. Endocrinol. 5, 1755ff (1991); Elliston *et al.*, JBC 267, 5193-5198 (1992)).

The carboxyl terminus of the hPR polypeptide as shown in Fig. 16 comprises a progesterone binding domain (PBD) but also contains sequences responsible for the association with heat shock proteins and receptor dimerization. The hinge region provides a flexible link between the DNA-binding domain (DBD) and the PBD but is also thought to contain elements for receptor dimerization as well as nuclear localization. Binding of the hPR to its corresponding target sites at the chromosomal DNA (PREs, Progesterone Responsive Elements) is known to be mediated by the DBD. The remaining N-terminal trans-activation domain (TAD) consists of regions specific for the *in vivo* function of the hPR as a transcriptional gene activator.

Even though the N-terminus also seems to contribute directly to the homodimerization of hPR after progesterone binding, it has been

demonstrated that a fragment comprising only the hinge region and the PBD was the minimal C-terminal fragment to mediate progesterone dependent hPR-hPR-interaction (Tetel *et al.*, Mol. Endocrinol. 11, 1114ff. (1997). It is believed that genetically engineered hPR polypeptides lacking either in part or completely the TAD (amino acids 1 to 556) might be expressed as structurally stable and fully soluble dimers in the presence of progesterone. Complexes with such a truncated hPR (provided that said truncated hPR exhibits DNA-binding activity as well as progesterone-binding activity) may functionally replace the complexes with the full length form of the described recombinant hPR-A or hPR-B proteins, since still mediating the contact between the plasmid DNA and the progesterone. Thus, the hPR in embodiments (2) and (6) to (16) of the invention preferably is a PR comprising nucleic acids 557 to 933 of natural hPR shown in SEQ ID NO: 18.

Effective expression of such a truncated version of hPR is possible in the baculovirus system but also in other eukaryotic expression systems, such as cultivated mammalian cells or yeast cells. Furthermore, also an *E. coli* overexpression strain is a possible system for the production of those polypeptides. In this case, the fusion of such a truncated hPR-version to a suitable polypeptide sequence, e.g. a histidine containing sequence or the GST (glutathion S- transferase) protein, might be helpful to overcome insolubility problems as well as to facilitate the isolation and purification of the expressed protein.

Mutated versions of these receptors and derivatives thereof, that still retain the function of the receptors to bind a ligand and thereby become activated and bind DNA and regulate transcription, may also be employed in the invention. Such derivative may be a chemical derivative, variant, chimera, hybrid, analog, or fusion.

The third component of the gene transfer system of the invention is the hormone. The hormone in the agent of embodiment 2 and in the composition of matter of embodiment (6) include synthetic and natural hormones, preferably steroid hormones, such as estrogen, testosterone, glucocorticoid, androgen, thyroid hormone, and progesterone or derivatives thereof. These are widely available. Progesterone is most preferred. For example, natural micronized progesterone is the preferred progesterone from which has been marketed in France since 1980 under the trademark of UTROGESTAN® and is still available in Germany under the trademark UTROGEST®. Its properties are similar to the endogenous progesterone, in particular, it has antiestrogen, gestagen, slightly antiandrogen and antimineralocorticoid properties. The natural micronized progesterone in said marketed products is dispersed in a matrix as described hereinbelow.

The above micronized progesterone has advantages that make it a suitable carrier for genes or nucleic acid constructs to target cells. Specifically, the synergistic effect of the double process of micronization and suspension in long-chain fatty acids residues of an oil results in increasing progesterone absorption. It has been demonstrated that after oral administration of 100 mg of UTROGESTAN®, peak plasma progesterone levels were obtained after 1-4 hours in most cases (Padwick, M. L., et al., *Fertil. Steril.*, Vol. 46, 402, 1986). Later on, the levels declined substantially, although they were still elevated at 12 hours. Even at 84 hours the levels were slightly higher than baseline. A U.S. kinetic study confirmed earlier work demonstrating the bioavailability of oral micronized progesterone. They showed a peak effect at 2 hours followed by rapid decrease in plasma progesterone level (Simon, J. A., et al., *Fertil. Steril.*, Vol., 60, 26, 1993).

A further advantage of using progesterone as a carrier is the low level of disadvantageous side effects. Orally administered progesterone adversely affects neither plasma lipids (Jensen, J. et al., Am. J. Obstet. Gynecol., Vol. 156, 66, 1987) nor carbohydrate metabolism (Mosnier-Pudar, H. et al., Arch. Mal. Coeur, Vol 84, 1111, 1991). Further, progesterone does not affect liver enzymes (ASAT, ALAT, AFOS), sex-hormone binding-globulin (SHBG) synthesis or HDL-cholesterol levels at daily doses of 200 mg and 300 mg. Although the plasma levels of deoxycorticosterone may increase substantially during UTROGESTAN® treatment, there are strong indications that the mineralocorticoid effects of this progesterone metabolite are completely counteracted by the anti-mineralocorticoid effects of progesterone itself. This is apparent from a comparative study (Corvol, P., et al., In: Progesterone and progestins. Raven Press, New York, 179, 1983) in which oral UTROGESTAN® was capable of antagonizing the mineralocorticoid effects of 9- $\alpha$ -fluorohydrocortisone.

In the agent of embodiment (2) and in the composition of matter of embodiment (6) of the invention the molar ratio of HRE (or SRE/or PRE) within the nucleic acid construct to hormone receptor is preferably from 1:1 to 1:10, more preferably from 1:2 to 1:5. On the other hand, the molar ratio of hormone to hormone receptor is preferably at least 1000:1, more preferably at least 10000:1. Thus, the hormone is present in a large excess relative to the hormone receptor and the HRE, which is desirable in view of the ability of the hormones to transfer nucleic acid constructs through cell membranes.

The skilled artisan will appreciate that the agent of embodiments (1) and (2) and the pharmaceutical composition of embodiment (10) may contain other components capable of assisting in introducing the nucleic acid into a cell for the purpose of gene therapy (matrix compounds). Specifically, the agent and the composition, especially

the hormone component thereof, may contain the following matrix compounds: glucose and related compounds (such as D-sorbitol, D-mannitol); solubilizing adjuvants (such as alcohols, e.g., ethanol); polyhydric compounds such as glycerine, polyethylene glycol and polypropylene glycol; nonionic surface active compounds, ionic surface active compounds such as lecithin; oily compounds such as sesame oil, peanut oil soybean oil, corn oil, etc.; starches and their derivatives such as cyclodextrines and hydroxyalkylated starches; stabilizers such as human serum albumin, preservatives such as benzyl alcohol and phenol; and the like. The preferred matrix contains  $\beta$ -cyclodextrine, glycerine, lecithin and/or corn oil. For example, the pharmaceutical composition of hormone-hormone receptor nucleic acid complex of the invention may be provided orally to humans or animals as a gelatin capsule. Progesterone therein (preferably in micronized form) could be present in a concentration of 50 to 1000 mg, preferably 200 - 300 mg dissolved in a 35 % or 40 %  $\beta$ -cyclodextrin solution or in corn oil or glycerol with peanut oil together with lecithin.

Alternatively, when - due to the selection of appropriate matrix components - the pharmaceutical composition is in a pasty, gel-like form, it may be provided topically.

The nucleic acid construct of embodiments (1) to (16) of the present invention may - aside from the transgene and the HREs, SREs, or PREs already disclosed above - further contain promoter, enhancer, and/or silencer sequences. The promoter may be ubiquitous or tissue-specific. Of the ubiquitous promoters, the CMV promoter is most preferred. However, a tissue-specific promoter is preferred over a ubiquitous promoter. For example, the tissue-specific promoters envisioned for the instant invention include  $\alpha_1$ -antitrypsin (further promoters).



The nucleic acid construct may further comprise additional sequences such as the ampicillin resistance gene. Other reporter sequences known to the skilled artisan may also be included, such as, for example, the green fluorescent protein (GFP), luciferase,  $\beta$ -galactosidase or chloramphenicolacetyltransferase (CAT). As an enhancer sequence, the SV40 intron and SV40 Poly A are most preferred. The nucleic acid construct may further contain inducible promoters such as, for example, a MMTV (Mouse Mammary Tumor Virus) promoters inducible via glucocorticoides and Ecdyson-inducible insect promoters.

A preferred nucleic acid construct contains sequentially from the 5' to the 3' end: a PRE, a CMV promoter, a gene of interest, SV40 Intron and SV40 poly A enhancer sequence, and an ampicillin resistant gene. Further PREs are evenly distributed on the vector backbone.

The nucleic acid construct may further contain origin of replication sequences (especially eukariotic origin of replication sequences), elements for gene targeting, integrational sequences (e.g., AAV-ITR, transposon IS), 3'-UTR, "switch" systems (e.g., TET system, Cre/loxP or Flp/ftr system).

The transgene may be chosen from those encoding proteins lacking in a variety of genetic disorders or involved in conditions related to inappropriate responses to hormones, for example, hormone-dependent cancers such as breast, ovarian, and endometrial cancers and prostate cancer. The transgene may also be used to replace a defective gene resulting in such genetic disorders as hemophilia, von Willebrand disease, and cystic fibrosis. The transgene includes mutations of such gene or a gene encoding a fusion product. The nucleic acid construct of the present invention may comprise more than one transgene.

In particular, the transgene may replace genes for a blood clotting factor, and preferably a human blood-clotting factor. The genes encoding factor VIII and factor IX (shown in Fig. 2, SEQ ID NO: 2), involved in hemophilia A and B, respectively, are good candidates for the invention. Other candidates include the gene encoding von Willebrand factor, factor IV, factor X, or protein C.

Other useful transgenes include, but are not limited to, hormone genes such as the genes encoding for insulin, parathyroid hormone, luteinizing hormone releasing factor (LHRH),  $\alpha$  and  $\beta$  seminal inhibins and human growth hormone; hormone receptor genes such as the glucocorticoid receptor, the estrogen receptor, the progesterone receptor, the retinoic acid receptor; growth factors such as vascular endothelial growth factor (VEGF), nerve growth factor, epidermal growth factor; enzyme genes; genes encoding cytokines or lymphokines such as interferons, granulocytic macrophage colony stimulating factor (GM-CSF), colony stimulating factor-1 (CSF-1), tumor necrosis factor (TNF), and erythropoietin (EPO); genes encoding inhibitor substances such as  $\alpha_1$ -antitrypsin, and genes encoding substances that function as drugs, e. g., genes encoding the diphtheria and cholera toxins, ricin or cobra venom factor. Also, antisense sequences may be administered as genetic material.

Another aspect of the present invention is vectors comprising the nucleic acid constructs of embodiment (3) of the present invention. These vectors may be used in the composition matter of embodiment (6) of the present invention. Preferably, however, the nucleic acid sequence for use in the invention is circular rather than linear. The vectors may be capable of expressing the nucleic acid in the nucleic acid construct transiently or permanently (including episomally). As noted above, the nucleic acid construct therein may further contain additional elements.

The composition of matter of embodiment (6) of the invention can be prepared by admixing the nucleic acid construct with the hormone receptor and the hormone. Preferably, an aqueous solution of nucleic acid construct was added to the oily suspension containing the hormone at ambient temperature under stirring.

Embodiment(s) of the invention relates to transfected and transformed cells or transgenic organism comprising these vectors and/or nucleic acid constructs. Within the scope of this invention, a transfected cell is one in which foreign DNA has been incorporated. Methods of transfection may include microinjection,  $\text{CaPO}_4$  precipitation, electroporation, liposome fusion, or gene gun.

Transformation refers to introducing genetic material into a cell, such as the vectors or nucleic acid constructs of the invention, rendering the cell transiently or permanently altered so that the cell expresses a specific gene product or is otherwise altered in its expression. Transformation may be achieved by *in vivo* or *in vitro* techniques, although *in vivo* transformation is preferred.

A further embodiment of the present invention is pharmaceutical compositions comprising a therapeutically effective dose of the nucleic acid constructs of the invention and a hormone. The hormone is preferably a steroid, and most preferably, progesterone, as described above. The dose is dependent on the condition to be treated, the characteristics of the patient, and the result sought to be achieved. Determining dosage is within the realm of the skilled artisan.

The pharmaceutical composition (or, alternatively, the composition of matter, the nucleic acid construct, or the vector) of the present invention may be administered orally, intravenously, intramuscularly, subcutaneously, topically, through mucosa (including buccal, nasal spray) or by gene gun. Oral administration (of a

micronized hormone dispersion) is preferred. Delivery may be systemic or directed at certain tissue.

The invention further includes a method of introducing into a cell a nucleic acid construct encoding a gene of interest, e.g., a human blood-clotting factor, to express the blood-clotting factor in the cell. In this method, the nucleic acid encoding a human blood-clotting factor is combined with a nucleic acid construct comprising at least one hormone responsive element (HRE), preferably a progesterone responsive element.

The mixture of nucleic acid bound to the hormone-hormone receptor complex together with an excess of hormone, preferably progesterone, will be used to introduce the nucleic acid into a cell by various methods known to the skilled artisan and outlined above. The cell-uptake will be stimulated by the interaction of the hormone with the cell membrane. The hormone or steroid interacts with the lipid bilayer of the cell membrane not only through membrane perturbation but also through activation of certain hormone- or steroid-sensitive membrane receptors. This has been demonstrated for progesterone and other steroids. Last but not least, it is known that hormones are able to cross the cell membrane by diffusion. In the present invention, the nucleic acid bound to the hormone-hormone receptor complex should be transported through the membrane during the process of diffusion or uptake.

Another aspect of the invention is a method of treating a blood clotting disorder by administering a therapeutically effective amount of the composition of matter of the invention to an organism. This method involves the administration and dosage considerations already discussed.

Embodiments (17) and (18) of the invention pertain to the use of a steroid hormone for preparing an agent for gene therapy and/or

gene transfer and to method for gene therapy and/or gene transfer which comprises administering a nucleic acid construct to an organism or to a cellular system, wherein the nucleic acid construct contains a transgene and is encapsulated in a steroid hormone. Suitable steroid hormones are enumerated hereinafter. The preferred steroid hormone in said embodiments of the invention is a natural micronized steroid hormone, in particular a natural micronized progesterone. In a preferred embodiment, the micronized hormone is solubilized/dispersed in a lipophilic matrix as described hereinafter.

Experiments have been performed to illustrate the technical aspects of the present invention. These experiments are described in examples 1 to 9 below. The skilled artisan will be readily recognize that the invention is not limited to these examples.

## Examples

### Example 1: Construction of Vectors

Production of the vector pTGFG1: The vector pUC19 (MBI Fermentas) was digested with XbaI, treated with Klenow enzyme and religated. This XbaI deleted vector was then digested with EcoRI, treated with Klenow enzyme and religated in order to delete the EcoRI site. For insertion of a XbaI site in the SacI site of this vector it was digested with SacI, treated with T4-polymerase, dephosphorylated with alkaline phosphatase and ligated with the XbaI-linker CTCTAGAG (Biolabs #1032). Another XbaI-site was inserted by digesting the newly produced vector with HindIII, treating it with Klenow, dephosphorylating it with alkaline phosphatase and ligating it with the XbaI-linker CTCTAGAG (Biolabs #1032). This vector was named pUC19/X.

In order to destroy the XbaI-site present in the vector phGFP-S65T (Clontech) this vector was digested with XbaI, treated with Klenow enzyme and religated resulting in the vector pGFP/0. A 2.3 kb fragment containing the GFP-Gene was isolated after digesting pGFP/0 with MluI, treating it with Klenow enzyme and digesting it with BamHI. This fragment was inserted into the multiple cloning site of the vector pUC19/X which was digested with SalI, treated with Klenow enzyme and digested with BamHI. The resulting vector was named pTGFG1 (Figure 2).

Starting with this vector all the vectors described in Table 1 were obtained. At the restriction sites for PstI, KpnI, EheI, EcoO109 and/or SapI a PRE(ds) was inserted giving rise to plasmids carrying the GFP gene and up to five PREs. By exchanging the GFP gene with a FIX gene a set of FIX expression plasmids were obtained. By excising the GFP gene the cloning vectors without a transgene were obtained.

Production of the insert PRE(ds): The oligonucleotides (Metabion) PRE-S (5'-GGG GTA CCA GCT TCG TAG CTA GAA CAT CAT GTT CTG GGA TAT CAG CTT CGT AGC TAG AAC ATC ATG TTC TGG TAC CCC-3'; SEQ ID NO: 3) and

PRE-AS (5'-GGG GTA CCA GAA CAT GAT GTT CTA GCT ACG AAG CTG ATA TCC CAG AAC ATG ATG TTC TAG CTA CGA AGC TGG TAC CCC-3'; SEQ ID NO: 4)

were hybridized and phosphorylated by kinase reaction, resulting in the insert PRE(ds).

Production of the vector pTGFG5: The vector pTGFG1 was digested with EcoO109I, treated with Klenow enzyme and dephosphorylated with alkaline phosphatase. It was then ligated with the PRE(ds) insert,

resulting in the vector pTGFG5 (Figure 3), i.e., a vector which carries a PRE at position C of Fig. 2.

Production of the vector pTGFG20: The vector pTGFG1 was digested with KpnI, treated with T4-polymerase and dephosphorylated with alkaline phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG7. This vector pTGFG7 was digested with PstI, treated with T4-polymerase and dephosphorylated with alkaline phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG11. Subsequently, pTGFG11 was digested with EcoO109I, treated with Klenow enzyme and dephosphorylated with alkaline phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG20 (Figure 4). This vector carries a PRE at positions A, B and D of Fig. 2.

Production of the vector pTGFG33: In a similar manner PRE(ds) were inserted at the restriction sites for PstI, KpnI, EheI, EcoO109 and SapiI in vector pTGFG1 giving rise to the plasmid pTGFG33 (Figure 5), which is a vector that carries the GFP gene and five PREs, one each in position A, B, C, D, E (Figure 2).

Production of the vectors pTGFG36, pTGFG53 and pTGFG64: The vector pUC19 (MBI Fermentas) was digested with SalI, treated with Klenow enzyme and dephosphorylated with alkaline phosphatase. It was ligated to the NotI-linker GCGGCCGC (Biolabs # 1045), resulting in the vector pUC19/N.

A 1.4 kb fragment containing the open reading frame of the human clotting factor IX, isolated from a human cDNA library (see example 2), was inserted into the PstI-site of the vector pUC19/N which was digested with PstI, treated with T4-polymerase and

dephosphorylated with alkaline phosphatase. From the resulting vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with Hind III and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG5 resulting in the vector pTGFG36 shown in Figure 6. This vector is a preferred one for delivery of Factor IX into the cell, and its DNA sequence is provided in Figure 9 (SEQ ID NO: 1).

In a similar manner plasmids pTGFG53 and pTGFG64 (shown in Figures 7 and 8) were obtained by exchanging the GFP gene in plasmids pTGFG20 and pTGFG33 by the FIX gene.

Production of the insert ALLG(ds): The oligonucleotides (Metabion) ALLG1/1 (5'-AGC TTG ACC TCG AGC AAG C-3') (SEQ. ID NO: 5) and ALLG2 (5'-GGC CGC TTG CTC GAG GTC A-3') (SEQ. ID NO: 6) were hybridized and phosphorylated by kinase reaction, resulting in the inserts ALLG(ds). The insert ALLG (ds) was constructed to introduce into the vector of choice a sequence with a multiple cloning site for the possible introduction of other transgenes.

Table 1 gives an overview of the available vectors with different transgenes and a different number of PREs in various positions. The positions of the PREs are given according to Figure 2. For the underlined vectors a map is provided (Figures 3 to 8).

**Table 1: Vectors of the invention**

| Plasmid       | Trans-gene | PRE | Plasmid        | Trans-gene | PRE | Plasmid        | Trans-gene | PRE |
|---------------|------------|-----|----------------|------------|-----|----------------|------------|-----|
| pTGFG0        | --         | --  | pTGFG18        | GFP        | BDE | pTGFG34        | FIX        | E   |
| <u>pTGFG1</u> | GFP        | --  | pTGFG19        | GFP        | BCD | pTGFG35        | FIX        | A   |
| pTGFG2        | FIX        | --  | <u>pTGFG20</u> | GFP        | ABD | <u>pTGFG36</u> | FIX        | D   |
| pTGFG3        | GFP        | E   | pTGFG21        | GFP        | CDE | pTGFG37        | FIX        | C   |



|         |     |    |         |     |       |         |     |       |
|---------|-----|----|---------|-----|-------|---------|-----|-------|
| pTGFG4  | GFP | A  | pTGFG22 | GFP | ACD   | pTGFG38 | FIX | B     |
| pTGFG5  | GFP | D  | pTGFG23 | GFP | ABC   | pTGFG53 | FIX | ABD   |
| pTGFG6  | GFP | C  | pTGFG24 | GFP | ABE   | pTGFG64 | FIX | ABCDE |
| pTGFG7  | GFP | B  | pTGFG25 | GFP | ACE   | pTGFG66 | --  | A     |
| pTGFG8  | GFP | BC | pTGFG26 | GFP | ADE   | pTGFG67 | --  | D     |
| pTGFG9  | GFP | BE | pTGFG27 | GFP | BCE   | pTGFG68 | --  | C     |
| pTGFG10 | GFP | BD | pTGFG28 | GFP | BCDE  | pTGFG69 | --  | B     |
| pTGFG11 | GFP | AB | pTGFG29 | GFP | ACDE  | pTGFG82 | --  | ABD   |
| pTGFG13 | GFP | CD | pTGFG30 | GFP | ABCE  | pTGFG95 | --  | ABCDE |
| pTGFG14 | GFP | AC | pTGFG31 | GFP | ABDE  |         |     |       |
| pTGFG15 | GFP | DE | pTGFG32 | GFP | ABCD  |         |     |       |
| pTGFG16 | GFP | AD | pTGFG33 | GFP | ABCDE |         |     |       |

For the DNA sequence of pTGFG 36, pTGFG 53, pTGFG 64, pTGFG 67, pTGFG 82 and pTGFG 95, see SEQ ID NOs: 1 and 13 to 17, respectively.

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## Example 2: Isolation of Human Factor IX cDNA

Factor IX cDNA was amplified from human liver cDNA (Clontech) using two primers overlapping the start and termination codon of the factor IX open reading frame resulting in a 1387 bp fragment containing the entire open reading frame. Restriction sites for EcoRI (upstream) and BamHI (downstream) were included at the end of each primer to facilitate cloning. Amplification was performed with Pwo polymerase (Boehringer Mannheim) in 50 µl reaction volume [10 mM Tris HCl pH 8.85, 25 mM KCl, 5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>] with 30 incubation cycles at 96°C for 1 min, 60°C for 1 min, 72°C for 2 min, followed by a final extension step at 72°C for 10 min.

Reaction products were ligated into the EcoRI- and BamHI-sites of pUC19 and transformed into *E. coli* DH5-a. Positive clones were

selected. Sequences were confirmed by cycle sequencing (Amersham) from both ends with labeled primers (IR-700) and automated analysis on the LiCor sequencing system (MWG, Biotech).

The following primers were used :

- 5 GGAATTCCGCAAAGGTTATGCAGCGCGTGAACATGATCATGGC  
(upstream; SEQ. ID NO: 7)  
CGCGGATCCATTAAGTGAGCTTTGTTTTTTCCTTAATCC (downstream;  
SEQ. ID NO: 8)

### 10 **Example 3: Expression and Quantification of the Marker Protein GFP**

Method 1: HeLa cells were transfected by electroporation with plasmids pTGFG5 or pTGFG20. Transfected cells were harvested and  
15 the cell pellets were homogenized and lysed in a buffer containing phosphate buffered saline (pH 7.5) and 10 mM PMSF. The concentration of green fluorescent protein (GFP) in the cell homogenate was determined by competitive ELISA.

For this purpose, GFP was coated in a defined concentration on  
20 microtiter plates. Then, GFP samples were added in the presence of anti-GFP antibody. After several washing steps a labeled secondary antibody was added in order to trace the first antibody. The colorimetric reaction was measured photometrically (extinction). Generally, the more GFP was added the less antibody was left to bind  
25 the coated GFP. Thus, reduction of extinction corresponded to higher GFP concentration in the sample.

A concentration curve of GFP was determined by linear regression (Figure 11) using bovine serum albumin (BSA) as a reference. A mean value of 2.4 mg GFP/ml for pTGFG5 (1 PRE) and  
30 5.2 mg GFP/ml for pTGFG20 (3PREs) was found.

Figures 12 a-d show micrographs of HeLa cell cultures transfected with pTGFG5 (Fig. 12 a and b) and pTGFG20 (Fig. 12 c and d), respectively. Figures 12 a and c represent light microscopic views as controls, and Fig. 12 b and d show the corresponding cell patches in the fluorescent mode. Routinely, more than 50% of the cells expressed GFP, indicating very efficient transfection, the presence of only one PRE showing more efficient expression.

Method 2: 293 T cells were transfected with pTGFG 5, 20 and 33 using calcium phosphate method and fluorescence was detected with a fluorimeter (Labsystems, Extinction: 485 nm Emission: 520 nm). In the case of the mock transfection, non GFP-expressing DNA was used. Background indicates the fluorescence of the empty plate (96-well plate, Dynex, Immulon-4). The results are summarized in Fig. 13. Again the vector with just one PRE (pTGFG5) shows the highest expression.

#### **Example 4: Human Factor IX Quantification by ELISA Assay**

HeLa cells were transfected either by electroporation or using liposome reagent DOTAP (Boehringer Mannheim) with plasmids pTGFG36, pTGFG53 and pTGFG64. These plasmids contain the cDNA of human clotting factor IX. Recombinant human factor IX was secreted into the supernatant of the cell culture and quantified using a sandwich ELISA method.

0.11 M sodium citrate and 10 mM PMSF were added in order to prevent degradation of human factor IX. The enzyme-immunological in vitro assay "Asserachrom IX:AG" from Boehringer-Mannheim was used in order to determine the concentration of expressed human

clotting factor IX. The factor IX-standard from Octapharma AG was used as a standard in aqueous solutions of 28 IU/ml.

In six different transfection experiments, in which HeLa cells with plasmids containing human factor IX-cDNA (pTGFG36, 53 and 64) were transfected using either electroporation or lipid-transfection reagent (DOTAP, Boehringer Mannheim), a concentration range of 3-25 ng/ml human clotting factor IX was reached.

### Example 5: Production and Purification of hPR (A Form)

1. Cloning of the human progesterone receptor: The cloning was performed as follows: Total human RNA was isolated from human white blood cells or liver cells using cell lysis in guanidinium hydrochloride buffer and CsCl-density centrifugation.

For cloning of the hPR coding sequence, hPR specific cDNA was prepared and used for amplification of the hPR coding sequence in two fragments by PCR.

The following oligonucleotide primers were selected based on the published mRNA sequence (Genbank: NM\_000926 and X51730).

Oligonucleotides used were obtained from MWG, Ebersberg or Metabion, München. All primers used are listed 5' to 3', bases added to introduce restriction sites are in capital letters and restriction sites used for cloning are underlined.

hPGR-5'-primer: CGA GGA tcc agt cgt cat gac tga gc (SEQ ID NO: 9);

hPGR-3'-primer: GCA GAA TT cat tat aaa aac tca aga cct cat aat cct gac (SEQ ID NO: 10);

hPGR-internal primer (Sal I) 1: ctc ctc ggg gtc gac cct gg (SEQ ID NO: 11);

hPGR-internal primer (Sal I) 2: cca ggg tcg acc ccg agg ag (SEQ ID NO: 12).

Synthesis of cDNA was performed using 3 µg of total RNA and 200 pmol of the 3'-primer with SuperScript II reverse transcriptase (Gibco BRL). Reaction volume was 50 µl and buffer was used as recommended, supplemented with RNase Inhibitor and 10 mM DTT and 1 mM dNTPs. Before adding the enzyme, samples were heated to 80°C for 10 min, followed by 10 min at 72°C and 10 min at 42°C. SuperScript II RT was added at 42°C and reaction was continued for 15 min at 42°C, 15 min at 50°C and 1 h at 58°C.

The cDNA obtained from this synthesis reaction was used to amplify the hPGR coding sequence in two fragments by PCR. One fragment (5') with 5'-primer and internal primer 2 and one fragment (3') with 3' primer and internal primer 1. Reaction setup in 50 µl was : Pwo polymerase (Roche Diagnostics), buffer as supplied by Roche Diagnostics, supplemented with DMSO, 50 pmol of each primer and 0.2 mM dNTPs. Reaction conditions were: 10 min 96°C followed by 35 cycles of 1 min 96°C, 2 min at 59°C, 2 min 72°C and a final extension step at 72°C for 10 min.

PCR-products were purified by gel electrophoresis and digested with Sal I. The BamHI and Hind III sites introduced in the primer were not used to avoid cutting at two internal restriction sites of the hPR coding sequence. Both fragments were ligated into pBluescript SK+ vector cut with EcoRV through blunt end ligation into the vector and sticky end ligation through the internal Sal I site. Vectors containing the appropriate insert were identified by mini-prep, restriction digest and sequencing. The obtained vector was designated pTGhPR1.

2. Production of hPR (A-form): Initially, the gene for hPR-B inclusive its 3'-UTR was cut out from pTGh PR1 and cloned in frame in the multiple cloning site of the expression plasmid pFASTBAC HTc (BAC-to-BAC Baculovirus Expression System, Life Technologies). This resulted in an expression cassette of a N-terminally histidine-tagged version of hPR-B under expression control of the viral polyhedrin promoter as shown below. A rTEV protease cleavage site is located between the six histidine residues and the initial methionine of the hPR-B reading frame, which allows removal of the histidine residues from the expressed protein. The N-terminal region of the expression cassettes is shown below.

MSYYHHHHHHDYDIPTTENLYFQ\*\*GAMGIRNST-hPR-gen  
           6 x His        \_\_\_\_\_  
                           spacer        \_\_\_\_\_  
   rTEV cleavage site

Amino acids are presented in the single letter code. The cleavage site of the rTEV protease is represented by \*\*

20

In order to generate the expression cassette for the truncated hPR-A form, the DNA sequence encoding for the amino acids between Met 1 and Met 165 of the hPR-B form was removed using a PCR-based strategy. Two primer pairs were designed which allowed amplification of either a DNA fragment just downstream of the start AUG of the hPR-B gene and a DNA-fragment just upstream of the AUG coding for Met 165, respectively. In a subsequent PCR reaction these two DNA fragments were annealed to each other at their homologous 3'-ends, and amplified using the outermost amplification primers. The resulting DNA-fragment was digested by EcoRI and Mlu I and the cleavage product was exchanged against the corresponding fragment of the

30

hPR-B expression cassette in the pFASTBAC HTc vector. Thereby the reading frame coding for an N-terminal histidine tagged version of the hPR-A polypeptide (94kDA) was restored.

This 6×His-tag was utilised for affinity purification of the protein by immobilized cobalt<sup>2+</sup> affinity chromatography on a TALON<sup>®</sup> resin (Clontech). The procedure, following the method of Boonyaratanakornkit et al. Mol. Cell. Biol.18, 4471 (1998), was as follows (all steps were carried out at 0 to 8°C):

Sf9 cells were cultivated in monolayer culture in serum free SF900 medium. Viral infection of the cells was done at a multiplicity of infection (MOI) of 5-8.

The harvesting was done 48 hours after infection with baculovirus containing the hPR expression cassette and lysed mechanically by homogenising in buffer A containing 20 mM Tris-Cl pH 8.0, 350 mM NaCl, 10 mM imidazol, 5% glycerol and a cocktail of proteinase inhibitors (Complete<sup>™</sup> EDTA-free, Roche Diagnostics, Penzberg, Germany). After a 10 min centrifugation at 10000 x g, supernatant originating from 10<sup>8</sup> cells was incubated for 1 h with 0,5 ml settled TALON<sup>®</sup> resin equilibrated in buffer A. TALON<sup>®</sup> was washed with 20 volumes of buffer A. hPR-A was eluted with 10 Vol buffer B, containing all ingredients of buffer A, but 100 mM imidazol. The eluate was concentrated 50-fold and dialysed against 100 volumes buffer C (PBS + 100 nM progesteron) by centrifugal ultrafiltration at a molecular exclusion size of 10 kDa (Centricon Plus-20 PL-10, Millipore, Eschborn, Germany).

3. Determination of identity, purity and yield of hPR-A: Purity and yield of the product were determined by application on denaturing reducing polyacrylamid- gelelectrophoresis according to Laemmli, U. et al., Nature 227, 680-685 (1970) and subsequent staining with

coomassie® blue R250. By this one-step procedure hPR-A was enriched to a final specific hPR content of 0.2 - 0.5 mg hPR/mg protein. As depicted in Figure 15, lane A, the final preparation consisted predominantly of two distinct protein species displaying  
5 apparent molecular masses of 94 and 74 kDa (Fig. 15, arrows).

Yield was estimated by parallel separation of standardised protein preparations. Data taken from a set of three separate experiments hint at a typical yield of 30 µg enriched hPR A-receptor per 10<sup>8</sup> cells.

10 Identity of hPR was determined by immunodetection of the product transferred to nitrocellulose by western blotting with mouse monoclonal antibodies directed against recombinant hPR (PR Ab-1, Oncogene, Cambridge, MA, USA).

The final product was transferred to nitrocellulose BA-83 and  
15 immunostained as described above. As presented in Figure 15, lane C, three major protein bands were detected, including the two dominant protein species described above. The smaller sized bands may display copurified proteolytic fragments of hPR.

Intracellular GFP from adherent cells was detected by a  
20 fluorimeter after media was taken off and PBS (colourless) was added. The results are summarized in Fig. 13.

### **Example 6: Clotting Activity of Human Clotting Factor IX from Transfected 293 T Cells**

25

A concentration range of 55 - 95 ng/ml human clotting factor IX has been reached by transfection of 293 T-cells with plasmids containing human factor IX-cDNA (pTGFG 36, 53, 64 and 2) in 11 different experiments using ELISA (Example 4).

30 Clotting activity was determined with a partial thromboplastin time



assay using Cephalin (phosphatidyl ethanolamine) activation with a manual coagulation instrument (ML-2, Instrumentation Laboratories). For the study, 100 µl undiluted supernatant from transfected 293 T-cells, 100 µl deficiency plasma (Progen) and 100 µl Cephalin (Instrumentation Laboratories) were incubated for 5 minutes at 37°C. Coagulation was started by adding 100 µl CaCl<sub>2</sub>. Sample coagulation time was compared to normal plasma.

| Number of cells<br>[ /ml] | Factor IX-concentration<br>[ng/ml] | Clotting time<br>[s] |
|---------------------------|------------------------------------|----------------------|
| 2,1 x 10 <sup>5</sup>     | 36                                 | 45                   |
| 8,7 x 10 <sup>5</sup>     | 20                                 | 79                   |

10 Normal plasma: 37 – 39 s  
Factor IX deficient plasma: 137 – 140 s

#### **Example 7: Analysis of an Additive Effect of Human Clotting Factor IX on the Clotting Time of Mice Blood**

15 1. Clotting time: Clotting activity was determined with a partial thromboplastin time assay using Cephalin (phosphatidyl ethanolamine) activation with a manual coagulation instrument (KC 4 A, Amelung).

20 For the study, 5 µl mouse blood, 20 µl deficiency plasma (Progen) and 100 µl physiological NaCl and 100 µl DaPPTin (Progen) were incubated for 2 minutes at 37°C. Coagulation was started by adding 100 µl CaCl<sub>2</sub>.

To analyse the additive effect, human clotting factor IX (housestandard, Octapharma) was added to the mouse blood and diluted 1:10 within the system. As it is shown in Figure 15, the additive effect of human clotting factor IX on clotting activity can be

detected up to a limit concentration of 0,07 mIU hFIX/ml (= 31,5 ng/ml).

2. ELISA: The addition of human clotting factor IX to the mouse blood was monitored by ELISA as described in Example 4. Citrate plasma was made out of mouse blood and human clotting factor IX was added in different concentrations.

| No. | Description                                | Concentration<br>[mIU/ml] hFIX added | Extinction at 405<br>nm [-] |
|-----|--|--------------------------------------|-----------------------------|
| 1.  | Mouse Citrate Plasma                       | 7                                    | 0,204                       |
| 2.  | Mouse Citrate Plasma                       | 2                                    | 0,130                       |
| 3.  | Mouse Citrate Plasma                       | -                                    | 0,099                       |
| 4.  | Control: 1.+2. Antibody<br>without antigen | -                                    | 0,096                       |
| 5.  | Control: 1. Antibody<br>without antigen    | -                                    | 0,072                       |
| 6.  | Control: 2. Antibody<br>without antigen    | -                                    | 0,085                       |
| 7.  | Substrate (ABTS)                           | -                                    | 0,072                       |

Mouse plasma without the addition of human clotting factor IX showed an extinction of 0,099 at 405 nm background. When added human factor IX in a concentration of 2 mIU/ml (= 9 ng/ml human factor IX) the detection limit is reached. It can be deduced that the antihuman factor IX antibodies used in the ELISA are not cross-reactive with mouse coagulation factor IX.

### Example 8: Cloning and Activity Testing of the Human Progesterone Receptor (hPR)

2. Activity Testing: The human progesterone receptor encoded in plasmid pTGhPR1 (s. Example 8.1 above) was tested for its physiological activity. In a functional form and after activation with a progestin like R5020 the receptor should be able to induce the expression of luciferase from a Mouse Mammary Tumor Virus (MTV) promoter.

To test this 293T cells were grown in phenol red-free DMEM supplemented with 10% charcoal-filtrated fetal calf serum and with or without 10 nM of R5020 (NEN) in 6 well plates. Transfections were performed by the calcium phosphate method using 2 µg of a pSG-hPR1 construct and pMTV-luc (Hollenberg et al., 1985, Cell 55, p899-906) per well. One day after transfection the cells were washed in PBS and the luciferase expression assayed with the Berthold luciferase kit according to the manufacturer's directions in a fluorimeter (Labsystems). The controls were as follows: R5020 was omitted (PR+MTV) and both plasmids alone were transfected with (PR+R5020, MTV+R5020) and without R5020 (PR, MTV). As positive control a plasmid with a CMV-driven luciferase gene was transfected (pCMV-luc).

As can be seen in Figure 19, there is a clear induction of luciferase expression when all the necessary elements are present, that is human progesterone receptor, progestin R5020 and the MTV-driven luciferase gene (PR+MTV+R5020). The error bars give the standard deviation of a threefold experiment, the readout is relative light units (RLU).

### Example 9: Oral Gene Transfer in *in vivo* Animal Experiment

Purpose of experiment: The object of this pilot study is to prove oral gene transfer in an *in vivo* animal experiment. Successful gene transfer is established by coagulation measurement: an additive effect of expressed human factor IX on the coagulation time of healthy murine whole blood is expected. The presence of expression of human factor IX in mouse blood is quantitated by ELISA.

Animals: The animals employed are 35 male C57BL/6J mice from Iffa Credo, France, with an initial age of 9 weeks and a weight of 23-33 g. The mice are kept in groups of 7 animals each in conventional test animal cages with wooden chips in the Institut für Experimentelle Onkologie und Therapieforchung der Technischen Universität München.

The animals are fed ad libitum with "Altrum Ratten und Mäuse Haltung" and are given tap water, also ad libitum.

The test animal cages are kept at an ambient temperature of 19-24°C and a humidity of 55-55%. The room is additionally provided with an automatic light supply which maintains a 12 hours rhythm.

The test animals are supervised by specialized staff.

#### Mixture of substances:

| Group | Hormone | Hormone receptor | Plasmid | Aqua dest. | Route of administration |
|-------|---------|------------------|---------|------------|-------------------------|
| 1. -  | -       | -                | -       | -          | -                       |
| 2. -  | 100 µl  | -                | 10 µg   | -          | oral                    |
| 3. -  | -       | -                | 10 µg   | 100 µl     | oral                    |
| 4. -  | -       | -                | 10 µg   | 50 µl      | i.m.                    |
| 5. -  | 100 µl  | 4.35 µg          | 10 µg   | -          | oral                    |

Plasmid and hPR: Theragene GmbH

Hormone: Utrogest® by Dr. Kade/Besins Pharma GmbH,  
Rigistr. 2, D-12277 Berlin

Aqua dest.: Aqua ad injectabilia Delta-Pharma GmbH, 72793  
Pfullingen

Esophageal sound: Vein catheter, diam. 0.5 x 0.9 mm,  
Lot 7077 G2221, B. Braun Melsungen AG, Western  
Germany

i.m. injection: Micro-Fine 12.7 mm, Becton Dickinson GmbH,  
Tullastr. 8-12, D-69126 Heidelberg

Course of experiment: The 35 mice were divided into 5 groups of 7 mice each. One group serves as a control, the second group was daily administered a total of 100 µl of hormone and plasmid via the gastrointestinal tract orally with an esophageal sound, the third group was daily administered a total of 100 µl of plasmid with aqua dest. orally with an esophageal sound, the fourth group was administered a total of 50 µl of plasmid with aqua dest. i.m. into the musculus quadriceps femoris, the fifth group was daily administered a total of 100 µl of hormone, hormone receptor and plasmid orally with an esophageal sound.

About 2-3 hours before the manipulation, the mice were prewarmed under a red light. Immediately before, during and after the manipulation, the mice were examined and supervised by a veterinarian.

Blood sampling from the mice was performed daily from the caudal artery of animals slightly sedated by inhalation anesthesia. For this purpose the artery was punctured with a disposable injection cannula (0.90 x 40 mm). Whole blood welling out of the puncture site (5 µl of blood) was immediately collected with an Eppendorf pipette.

Without further delay, the blood coagulation time in seconds was

determined using an Amelung-Koagulometer KC 4A by means of an aPTT assay (activated partial thromboplastin time). The blood coagulation analysis was always performed by the same person. Immediately after the blood sampling, the bleeding was stopped by compression.

Sedation of the mice was achieved by inhalation anesthesia (active substance: isoflurane: Forene, Abbott GmbH, 65205 Wiesbaden, Western Germany) in a whole body chamber.

The daily manipulation was performed through an overall period of 7 days. This was followed by a day (day 8 of experiment) without any manipulation, and at day 9 of experiment, again 5 µl of whole blood was withdrawn from the ventral caudal artery under anesthesia, and the coagulation time established as described above. Further, 0.5-0.75 ml of whole blood was collected intracardially using U-40 insulin syringes (Mikro-Fine 12.4 mm) filled with 50-75 µl of sodium citrate (3.1%), transferred into Eppendorf cuvettes, and about 100 µl of whole blood with citrate was reserved for PCR examination and stored in a cool environment. The remaining citrate blood was centrifuged for 10 min using a centrifuge 6000 rpm, 4°C, at 5000 rpm, and the plasma was recovered for the ELISA determination of the factor IX concentration.

Then, the animals were sacrificed using 0.5 ml Narkoren i.p. Immediately after the sacrificing, the animal bodies were dissected. The following organs were removed from the mice for an immunohistochemical examination: brain, spleen, liver, kidneys, testes, lungs, m. quadriceps femoris, heart, appendix; and frozen at -80°C.

Deviation from the scheduled experimental course: Due to the poor general condition of the mice in the course of the long-term administration series, the administration had to be interrupted at days

3 (except one mouse) and 5 for test group 2 (hormone and plasmid), at days 3 and 5 for group 5 (hormone, hormone receptor and plasmid), and two mice were additionally spared the administration of the reagents at days 2 and 7 of the experiment.

5 The poor general condition is accounted for by the hypnotic effect of the hormone progesterone. It causes the mice to sleep for about 24 hours without eating and drinking. This again has an adverse effect on the water balance of the mice, resulting in exsiccotic phenomena and apathic behavior. Therefore, the mice were prophylactically treated with  
10 a subcutaneous administration of 1 ml of 5% glucose solution (Delta Pharma GmbH, 72793 Pfullingen) and 1 ml of Ringer solution (Delta Pharma GmbH, 72793 Pfullingen) when the hormone was administered orally. Among the group which was orally administered hormone, hormone receptor and plasmid, two mice died at days 3 and 6,  
15 respectively; they were dissected.

Among the group which was orally administered hormone with plasmid, one mouse was found dead in its cage on day 8 of the experiment; it was also dissected.

The results are summarized in Figures 17 and 18. The statistical  
20 evaluations were performed according to the generalized linear model with repeated measurements (MANOVA with repeated measurements). In none of the test groups a non-linear course was observed. Therefore, the course was calculated by a simple representation of the linear increase or decrease, namely initial value minus final value per  
25 mouse. The particularly interesting difference between the control and the group "plasmid in the hormone with hormone receptor" (group 5) was examined using a T test for independent random samples.

Figure 17 shows the mean values of the calculated differences: In  
the control, for example, this difference was about 50 seconds. The  
30 vertical lines show plus and minus one standard deviation from these

values. The T test is based both on the differences between the mean values and on the degree of overlapping which can be seen from these lines: The larger the overlapping, the less is the significance of the mean value differences. Thus, the groups "control" and "plasmid and water i.m." (groups 1 and 5, respectively) are distinguished in a purely numerical way in the mean value, but the degree of overlapping is so high that these groups are not significantly different.

The only significant difference was between group 1 and 5: The decrease of the latter is significantly higher than that of the control ( $T = -2.357$ ; d.f. = 12;  $p < 0.05$ ).

The following Tables contain the concluding statistics and the results of the statistical tests (T test) performed on the differences between the mean values obtained in the course of the test:

15

## Group statistics

| ADMIN  | N | mean value | standard deviation | standard error of the mean value |
|--|---|------------|--------------------|----------------------------------|
| DIF control                                  | 7 | 47.3857    | 58.9946            | 22.2978                          |
| Hormone, hormone receptor and plasmid orally | 7 | 114.7571   | 47.3300            | 17.8891                          |



## Test for independent random samples

|     |                         | Levene test for equal variance |              | T test for equal mean values |        |                |                 |                              |                                       |         |
|-----|-------------------------|--------------------------------|--------------|------------------------------|--------|----------------|-----------------|------------------------------|---------------------------------------|---------|
|     |                         | F                              | Significance | T                            | df     | sig. (2-sided) | mean difference | standard error of difference | 95% confidence interval of difference |         |
|     |                         |                                |              |                              |        |                |                 |                              | lower                                 | upper   |
| DIF | variances are equal     | 0.026                          | 0.874        | -2.357                       | 12     | 0.036          | -67.3714        | 28.5869                      | -129.6570                             | -5.0858 |
|     | Variances are not equal |                                |              | -2.357                       | 11.461 | 0.037          | -67.3714        | 28.5869                      | -129.9833                             | -4.7596 |

The human F IX was also detectable in the treated mice of the "hormone-hormone reception and plasmid orally group using an Elisa as described in Example 4.

## Claims

1. Use of a nucleic acid construct comprising at least one hormone responsive element (HRE) and a transgene for preparing an agent for gene transfer.

2. The use of claim 1, wherein the at least one HRE is functionally linked to the transgene or not.

3. The use of claim 1 or 2, wherein the transgene is selected from the group consisting of genes encoding a blood clotting factor, hormone genes, hormone receptor genes, growth factors, enzyme genes, genes encoding cytokines or lymphokines, genes encoding inhibitor substances, genes encoding substances that function as drugs or vaccines, and antisense sequences.

4. The use of claim 3, wherein the transgene is a gene encoding a blood clotting factor and the agent is suitable for treating hemophilia.

5. The use of claim 4, wherein the human blood clotting factor is selected from the group consisting of factor VIII, factor IX, and von Willebrand Factor (vWF).

6. The use of claims 1 to 5, wherein the nucleic acid construct comprises 1 to 20, preferably 3 to 10 HRE(s).

7. The use of claim 1 to 5, wherein the at least one HRE is a steroid responsive element, preferably a progesterone responsive element (PRE).

8. The use of claim 5, wherein the HRE is a PRE and the blood clotting factor is factor IX, preferably the factor IX has a nucleotide sequence of 689 to 2071 of SEQ ID NO: 1.
- 5 9. The use of claim 5, wherein the HRE is a PRE and the blood clotting factor is factor VIII.
- 10 10. The use of claim 7 to 9, wherein the PRE has the double stranded DNA sequence comprised of the DNA sequences of SEQ ID NOs: 3 and 4.
- 15 11. The use of claims 1 to 10, wherein the construct further comprises functional DNA sequences selected from the group consisting of promoter sequences, enhancer sequences, silencer sequences, origin of replication sequences, integrational sequences, marker genes and switch sequences.
- 20 12. The use of claim 11, wherein the construct further comprises a tissue-specific promoter, preferably an  $\alpha$ -antitrypsin promoter.
13. The use according to any one of claims 1 to 12, wherein the agent further comprises a hormone-hormone receptor complex, preferably a steroid-steroid receptor complex.
- 25 14. The use of claim 13, wherein the molar ratio of HRE within the nucleic acid construct to hormone receptor is from 1:1 to 1:10, preferably 1:2 to 1:5, and/or the molar ratio of hormone to hormone receptor is at least 1000:1, preferably at least 10000:1.
- 30 15. The use of claim 13 or 14, wherein the receptor is a progesterone

receptor and the steroid is progesterone or a progesterone derivative.

16. The use of claim 15, wherein the progesterone is natural  
micronized progesterone solubilized in a lipophilic matrix system  
and/or the progesterone receptor is hPR-A, hPR-B or comprises the  
nucleotide sequence of 557 to 933 SEQ ID NO: 9.

17. A nucleic acid construct comprising at least one HRE and a  
transgene, wherein one of said at least one HREs is not functionally  
linked to the transgene.

18. The nucleic acid construct of claim 17, which is as defined in  
claims 3 to 12.

19. A vector comprising the nucleic acid construct of claim 17 or 18.

20. A transformed cell or transgenic organism comprising the nucleic  
acid construct as defined in claims 17 or 18 or the vector as defined in  
claim 19.

21. A composition of matter comprising a nucleic acid construct  
comprising at least one HRE and a transgene as defined in claim 17 or  
18 and/or a vector as defined in claim 19, said at least one HRE being  
coupled to a hormone-hormone receptor complex.

22. The composition of matter of claim 21, wherein the hormone-  
hormone receptor complex is as defined in claims 13 to 16.

23. The composition of matter of claim 21, wherein the transgene is a  
gene encoding a blood clotting factor.

24. The composition of matter of claim 21 wherein the blood clotting factor is factor IX.

5 25. The composition of matter of claim 21 wherein the blood clotting factor is factor VIII.

26. A method for preparing the composition of matter as defined in claim 21, which method comprises admixing the nucleic acid construct  
10 with the hormone receptor and the hormone.

27. A pharmaceutical composition comprising the nucleic acid construct of claim 17 or 18, the vector of claim 19, and/or the composition of matter of claim 21 to 25.  
15

28. The pharmaceutical composition of claim 27, which is suitable for gene transfer, preferably for treating hemophilia.

29. A method for gene transfer which comprises administering the agent as defined in claims 1 to 16, or the composition of matter as defined in claims 21 to 25 to an organism or to a cellular system.  
20

30. A method for delivering into an organism or into a cellular system a nucleic acid encoding a transgene to be expressed in the cells of the organism or the cells of the cellular system, which method comprises administering an agent as defined in claims 1 to 16 or composition of matter as defined in claims 21 to 25 to the organism or to the cellular system so that the hormone in the composition interacts with the cell membrane and therewith enhances diffusion and transport of the  
25

nucleic acid that is coupled to the hormone-hormone receptor complex across the membrane and into the cell.

31. The method of claim 30, wherein a nucleic acid encoding human  
5 factor VIII or factor IX is delivered into the cell.

32. A method of treating blood clotting disorders comprising  
administering a therapeutically effective amount of the composition of  
matter of claim 23 to an organism or to a cellular system.

10

33. A method of treating hemophilia B, comprising administering a  
therapeutically effective amount of the composition of matter of claim  
24 to an organism or to a cellular system.

15 34. A method of treating hemophilia A, comprising administering a  
therapeutically effective amount of the composition of matter of claim  
25 to an organism or to a cellular system.

35. Use of a steroid hormone for preparing an agent for gene transfer.

20

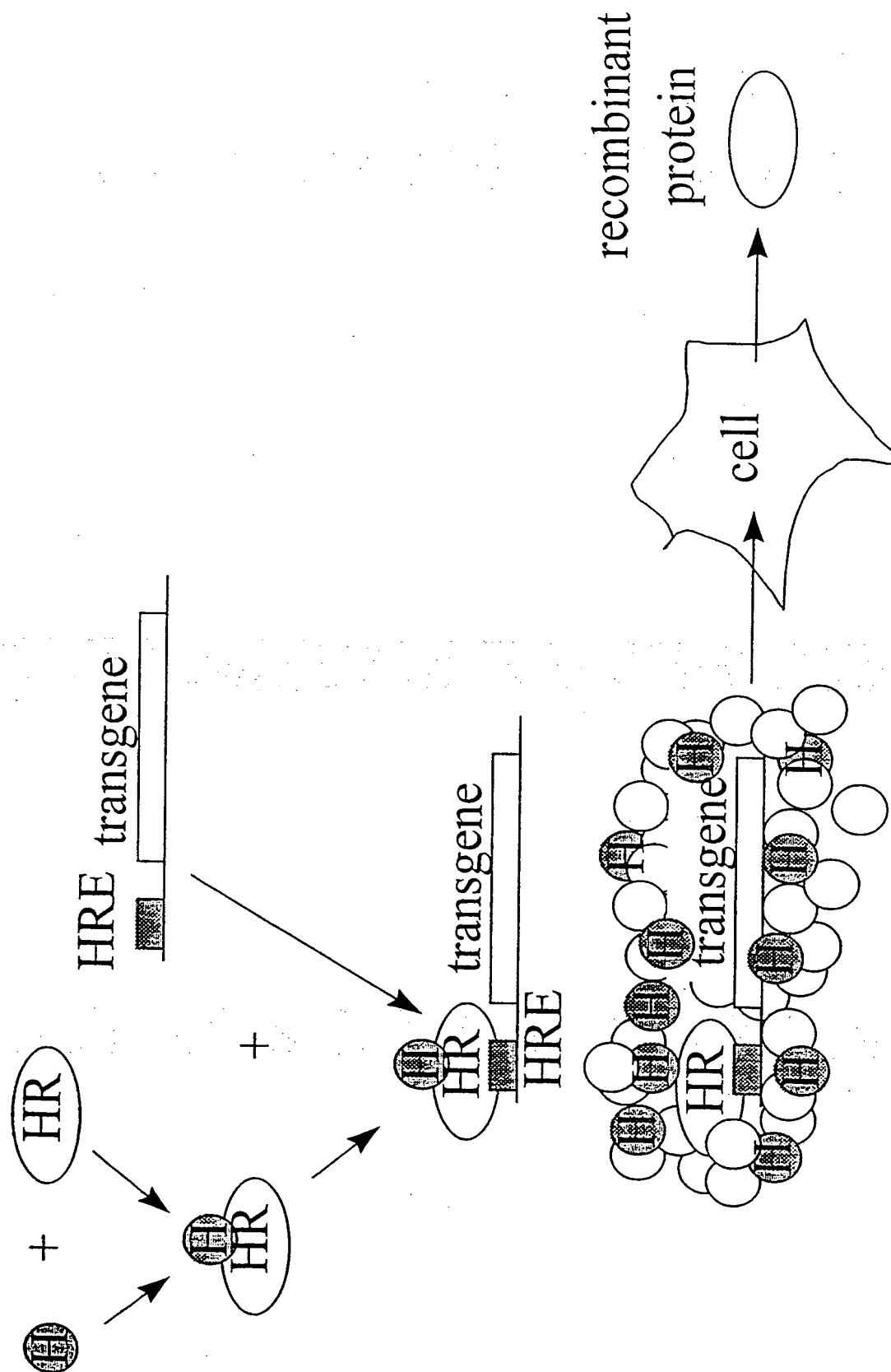
36. The use of claim 35, wherein the steroid hormone is a natural  
micronized steroid hormone, preferably natural micronized  
progesterone.

25 37. The use of claim 36, wherein the natural micronized steroid  
hormone is solubilized in a lipophilic matrix system.

38. A method for gene transfer which comprises administering a  
nucleic acid construct to an organism or to a cellular system, wherein

the nucleic acid construct contains a transgene and is encapsulated in a steroid hormone.

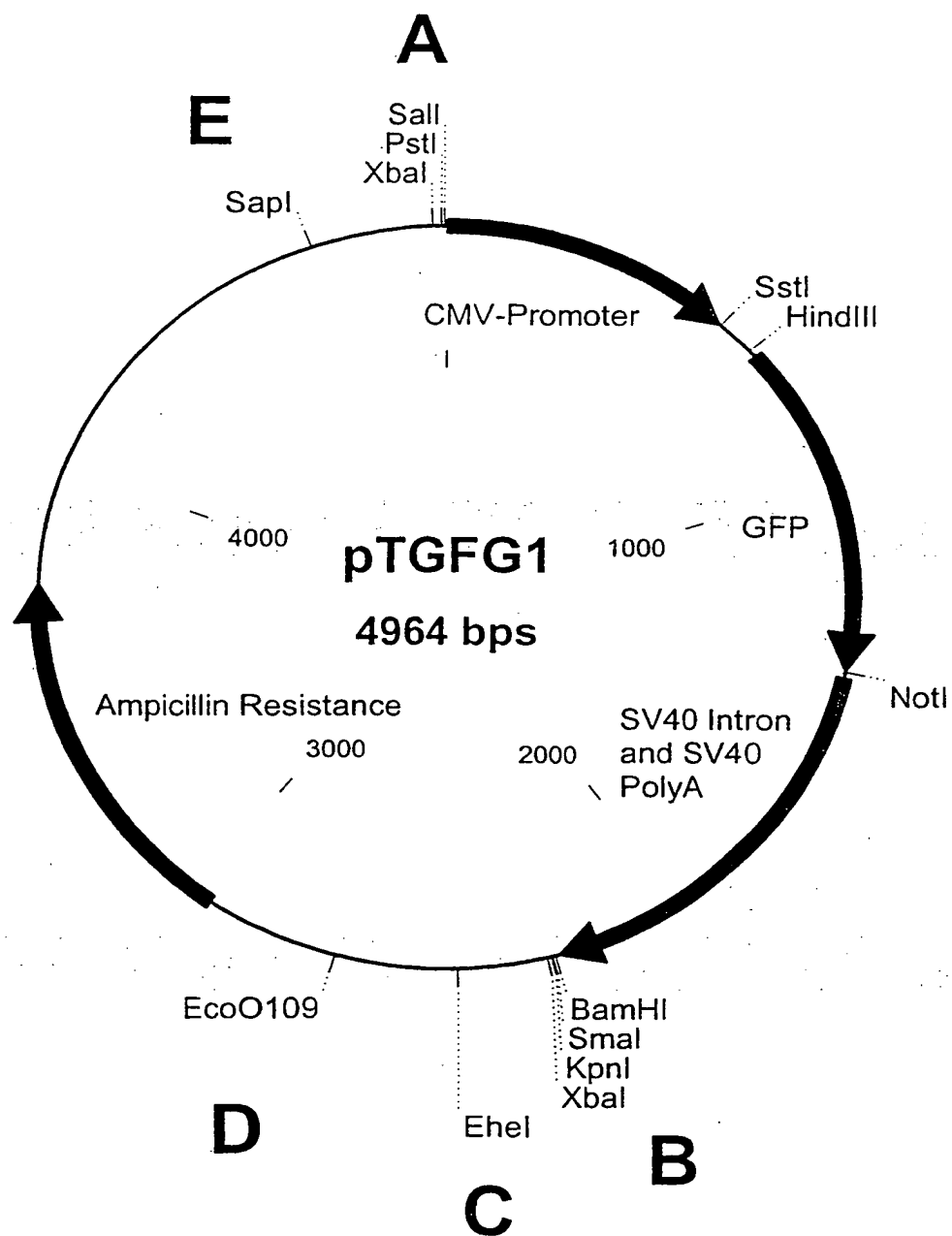
Fig. 1





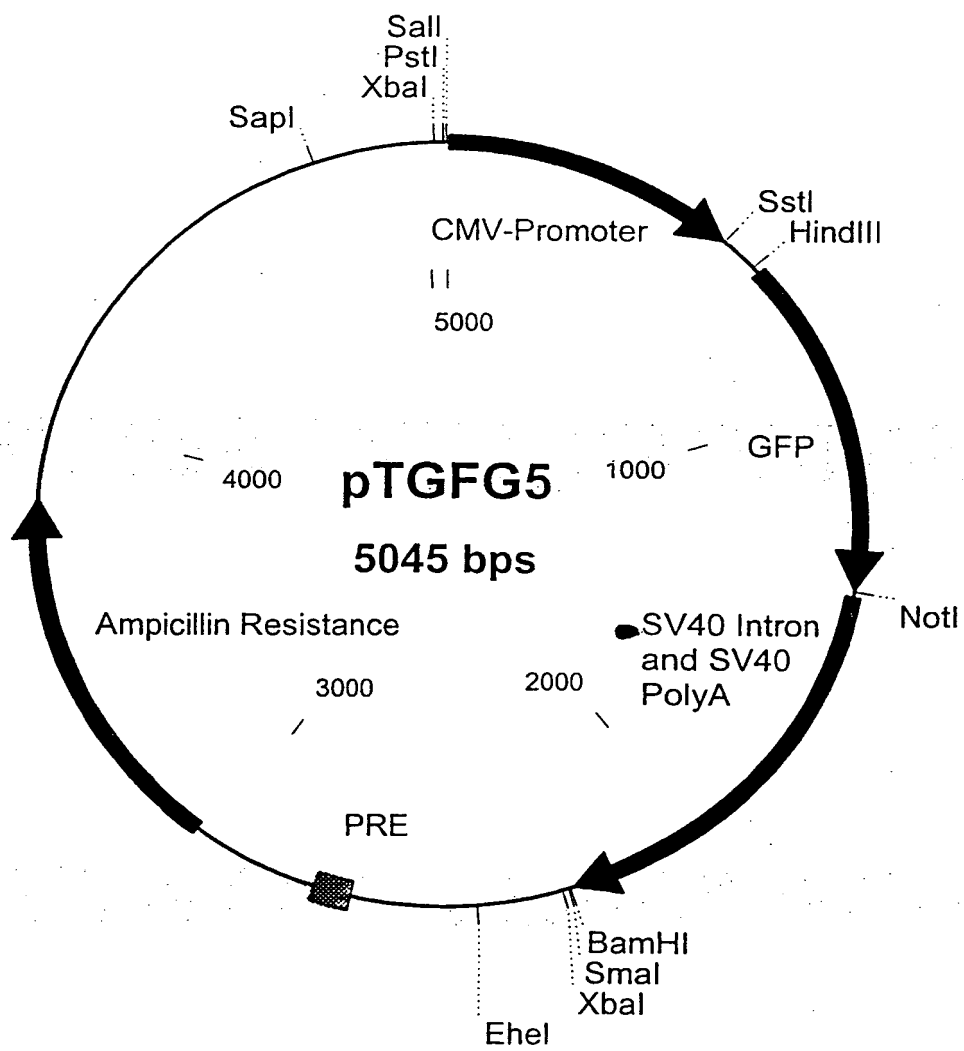
2/22

Fig. 2



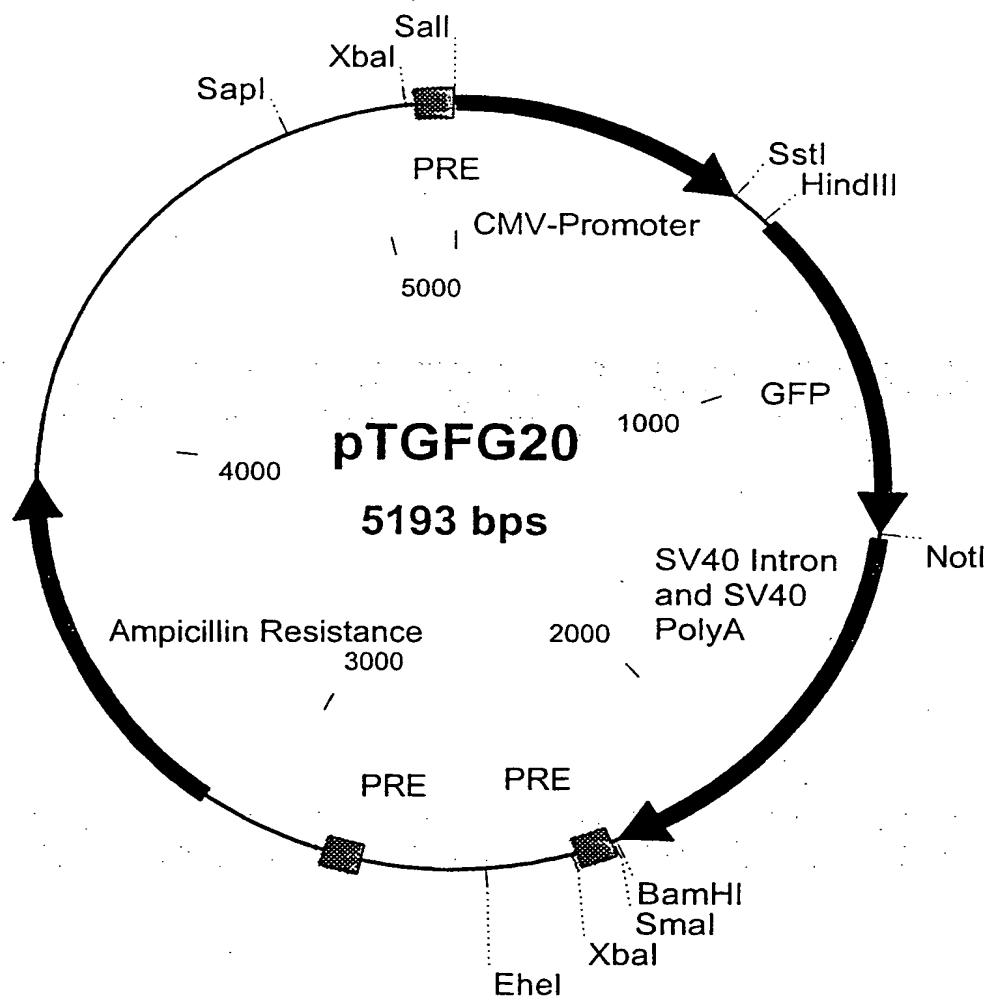
3/22

Fig. 3



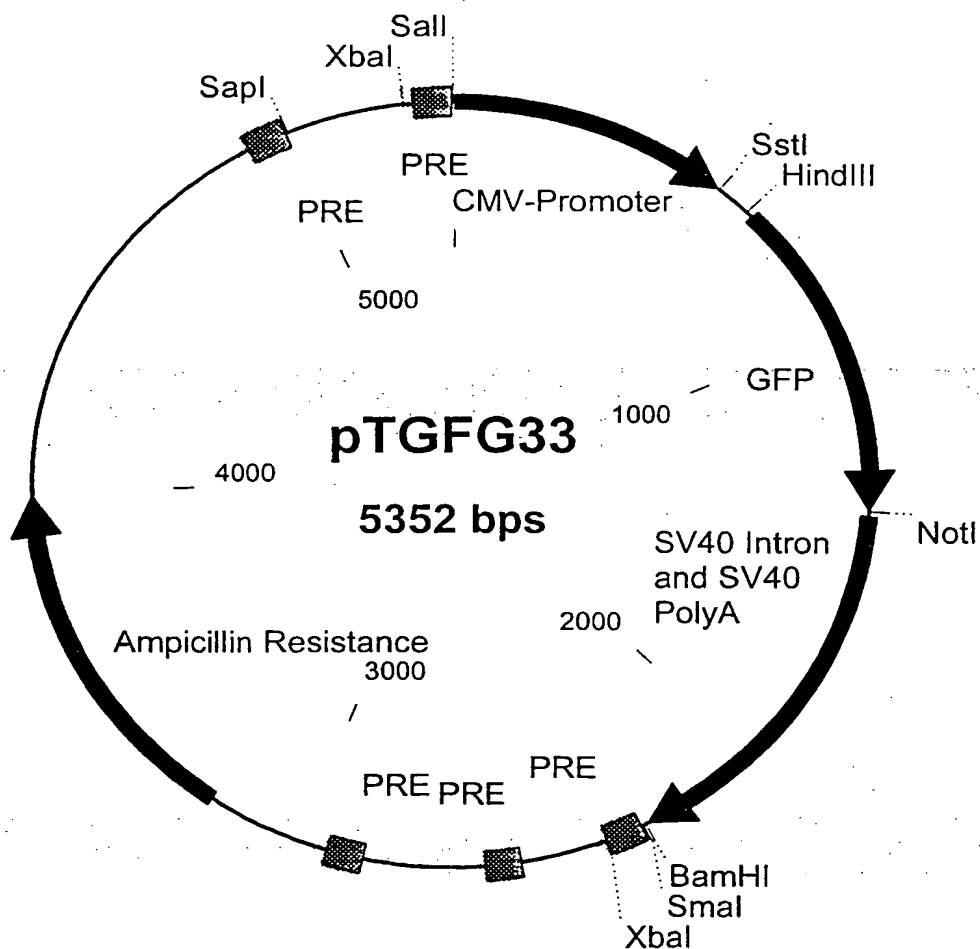
4/22

Fig. 4



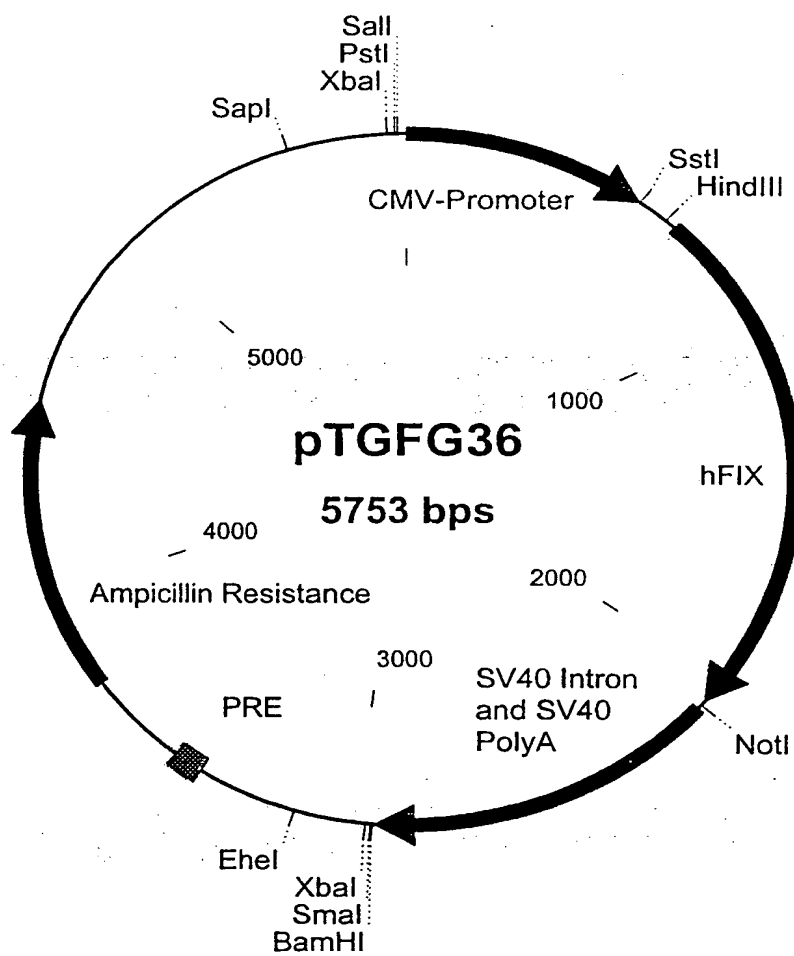
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Fig. 5



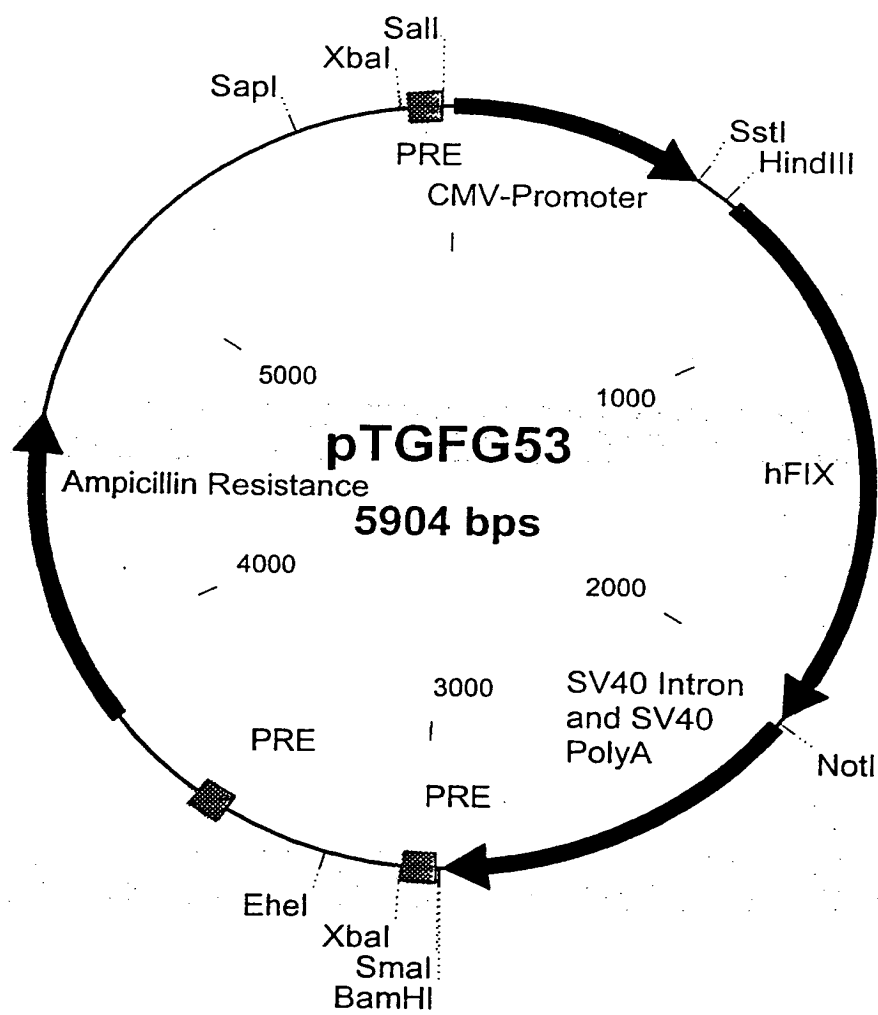
6/22

Fig. 6



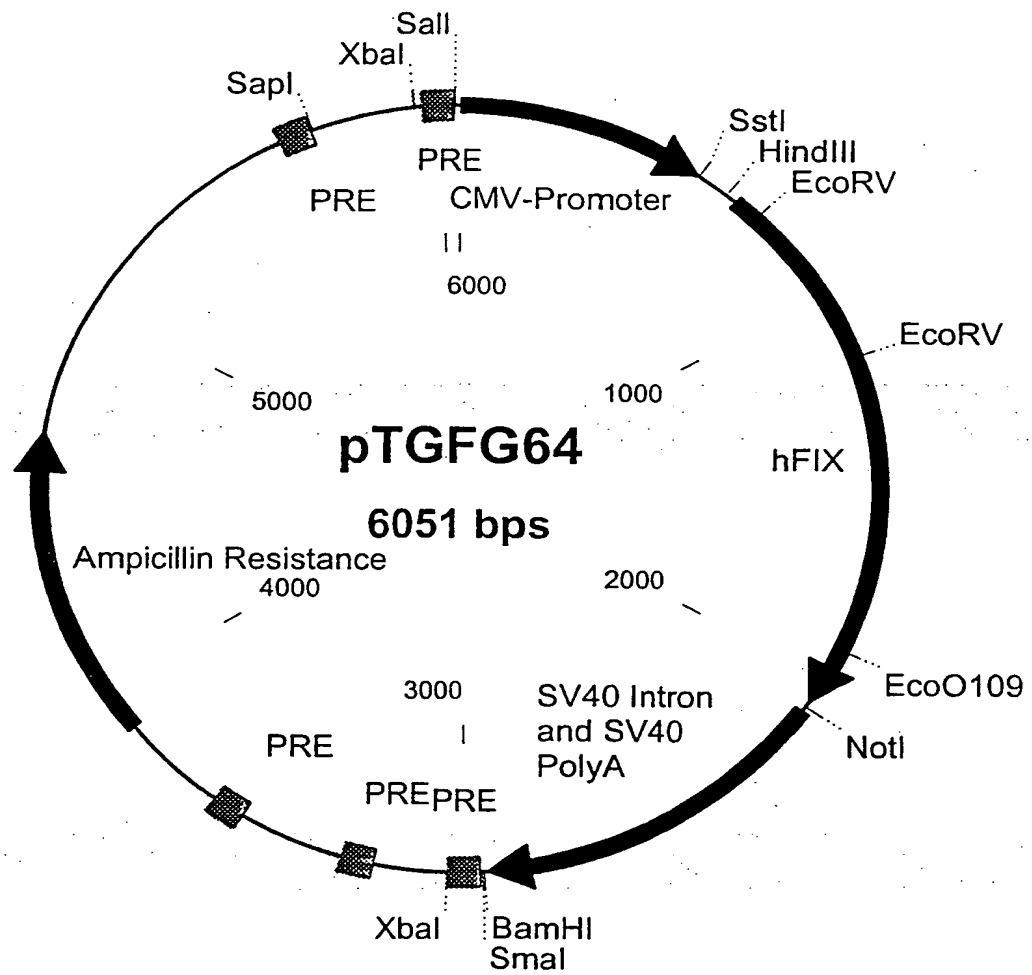
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Fig. 7



8/22

Fig. 8



9/22  
Fig. 9

CGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTTC  
CGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTA  
TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAG  
TACATCAAGTGTATCATATGCCAAGTACGCCCTTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAG  
TACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTG  
GCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTT  
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Fig. 9 (continued)

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AAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCC  
CTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAG  
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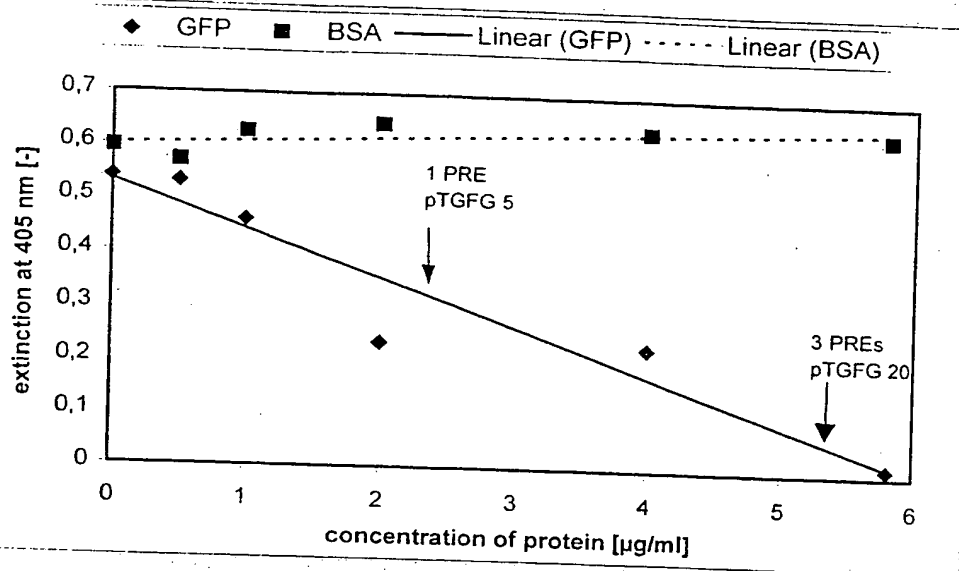
Fig. 10

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Gln | Arg | Val | Asn | Met | Ile | Met | Ala | Glu | Ser | Pro | Gly | Leu | Ile | Thr | 1   | 5   | 10  | 15  |
| Ile | Cys | Leu | Leu | Gly | Tyr | Leu | Leu | Ser | Ala | Glu | Cys | Thr | Val | Phe | Leu | 20  | 25  | 30  |     |
| Asp | His | Glu | Asn | Ala | Asn | Lys | Ile | Leu | Asn | Arg | Pro | Lys | Arg | Tyr | Asn | 35  | 40  | 45  |     |
| Ser | Gly | Lys | Leu | Glu | Glu | Phe | Val | Gln | Gly | Asn | Leu | Glu | Arg | Glu | Cys | 50  | 55  | 60  |     |
| Met | Glu | Glu | Lys | Cys | Ser | Phe | Glu | Glu | Ala | Arg | Glu | Val | Phe | Glu | Asn | 65  | 70  | 75  | 80  |
| Thr | Glu | Arg | Thr | Thr | Glu | Phe | Trp | Lys | Gln | Tyr | Val | Asp | Gly | Asp | Gln | 85  | 90  | 95  |     |
| Cys | Glu | Ser | Asn | Pro | Cys | Leu | Asn | Gly | Gly | Ser | Cys | Lys | Asp | Asp | Ile | 100 | 105 | 110 |     |
| Asn | Ser | Tyr | Glu | Cys | Trp | Cys | Pro | Phe | Gly | Phe | Glu | Gly | Lys | Asn | Cys | 115 | 120 | 125 |     |
| Glu | Leu | Asp | Val | Thr | Cys | Asn | Ile | Lys | Asn | Gly | Arg | Cys | Glu | Gln | Phe | 130 | 135 | 140 |     |
| Cys | Lys | Asn | Ser | Ala | Asp | Asn | Lys | Val | Val | Cys | Ser | Cys | Thr | Glu | Gly | 145 | 150 | 155 | 160 |
| Tyr | Arg | Leu | Ala | Glu | Asn | Gln | Lys | Ser | Cys | Glu | Pro | Ala | Val | Pro | Phe | 165 | 170 | 175 |     |
| Pro | Cys | Gly | Arg | Val | Ser | Val | Ser | Gln | Thr | Ser | Lys | Leu | Thr | Arg | Ala | 180 | 185 | 190 |     |
| Glu | Thr | Val | Phe | Pro | Asp | Val | Asp | Tyr | Val | Asn | Ser | Thr | Glu | Ala | Glu | 195 | 200 | 205 |     |
| Thr | Ile | Leu | Asp | Asn | Ile | Thr | Gln | Ser | Thr | Gln | Ser | Phe | Asn | Asp | Phe | 210 | 215 | 220 |     |
| Thr | Arg | Val | Val | Gly | Gly | Glu | Asp | Ala | Lys | Pro | Gly | Gln | Phe | Pro | Trp | 225 | 230 | 235 | 240 |
| Gln | Val | Val | Leu | Asn | Gly | Lys | Val | Asp | Ala | Phe | Cys | Gly | Gly | Ser | Ile | 245 | 250 | 255 |     |
| Val | Asn | Glu | Lys | Trp | Ile | Val | Thr | Ala | Ala | His | Cys | Val | Glu | Thr | Gly | 260 | 265 | 270 |     |
| Val | Lys | Ile | Thr | Val | Val | Ala | Gly | Glu | His | Asn | Ile | Glu | Glu | Thr | Glu | 275 | 280 | 285 |     |
| His | Thr | Glu | Gln | Lys | Arg | Asn | Val | Ile | Arg | Ile | Ile | Pro | His | His | Asn | 290 | 295 | 300 |     |

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Fig. 10 (continued)

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Asn | Ala | Ala | Ile | Asn | Lys | Tyr | Asn | His | Asp | Ile | Ala | Leu | Leu | Glu | 305 | 310 | 315 | 320 |
| Leu | Asp | Glu | Pro | Leu | Val | Leu | Asn | Ser | Tyr | Val | Thr | Pro | Ile | Cys | Ile | 325 | 330 | 335 |     |
| Ala | Asp | Lys | Glu | Tyr | Thr | Asn | Ile | Phe | Leu | Lys | Phe | Gly | Ser | Gly | Tyr | 340 | 345 | 350 |     |
| Val | Ser | Gly | Trp | Gly | Arg | Val | Phe | His | Lys | Gly | Arg | Ser | Ala | Leu | Val | 355 | 360 | 365 |     |
| Leu | Gln | Tyr | Leu | Arg | Val | Pro | Leu | Val | Asp | Arg | Ala | Thr | Cys | Leu | Arg | 370 | 375 | 380 |     |
| Ser | Thr | Lys | Phe | Thr | Ile | Tyr | Asn | Asn | Met | Phe | Cys | Ala | Gly | Phe | His | 385 | 390 | 395 | 400 |
| Glu | Gly | Gly | Arg | Asp | Ser | Cys | Gln | Gly | Asp | Ser | Gly | Gly | Pro | His | Val | 405 | 410 | 415 |     |
| Thr | Glu | Val | Glu | Gly | Thr | Ser | Phe | Leu | Thr | Gly | Ile | Ile | Ser | Trp | Gly | 420 | 425 | 430 |     |
| Glu | Glu | Cys | Ala | Met | Lys | Gly | Lys | Tyr | Gly | Ile | Tyr | Thr | Lys | Val | Ser | 435 | 440 | 445 |     |
| Arg | Tyr | Val | Asn | Trp | Ile | Lys | Glu | Lys | Thr | Lys | Leu | Thr |     |     |     | 450 | 455 | 460 |     |

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Fig. 11

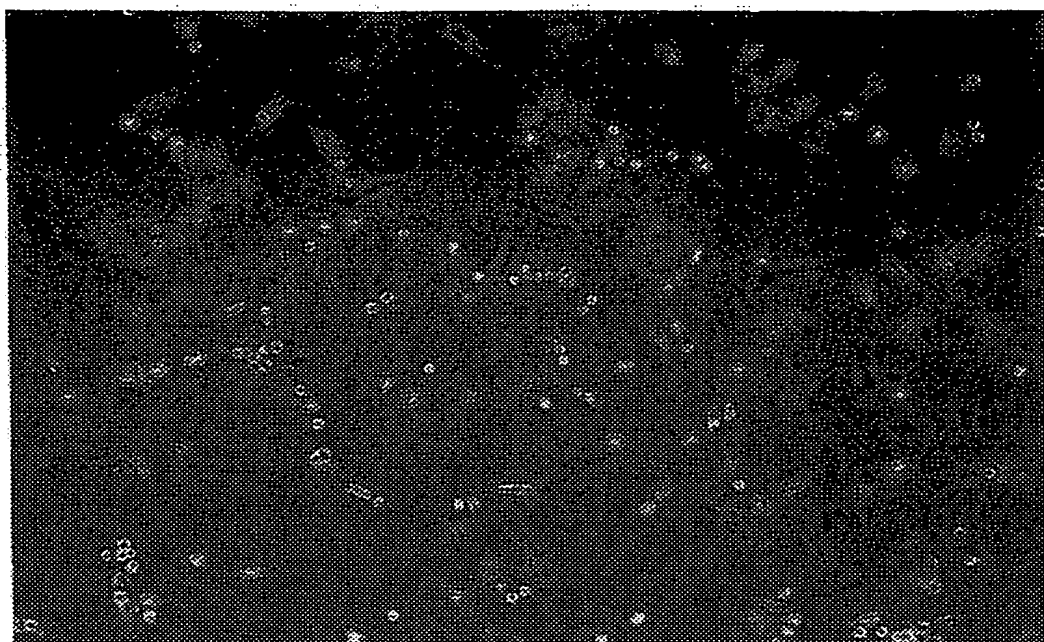


Fig. 12a



Fig 12 b

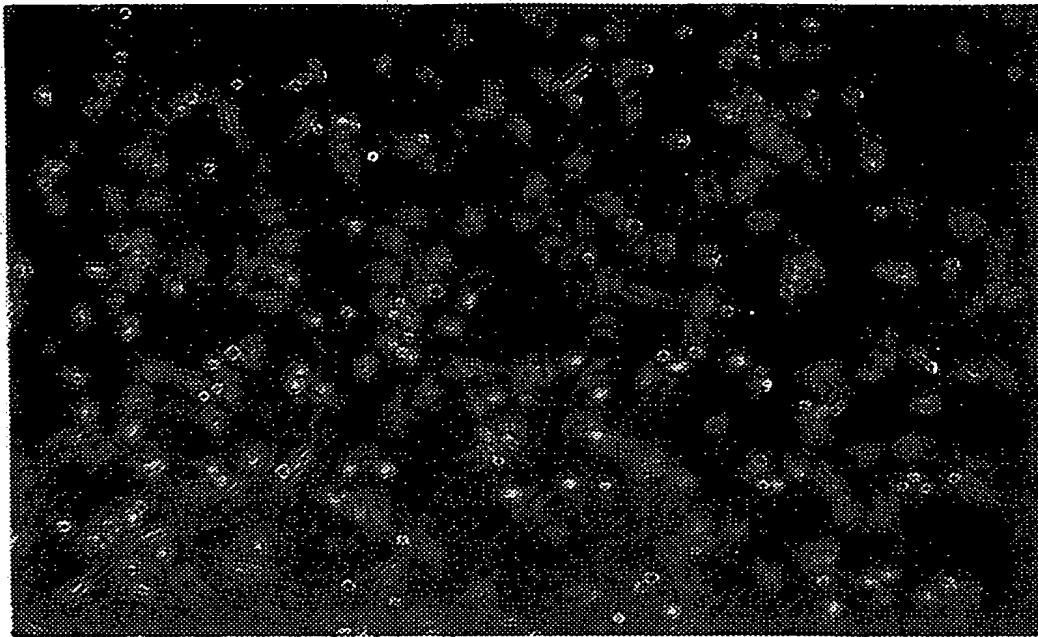


Fig 12 c

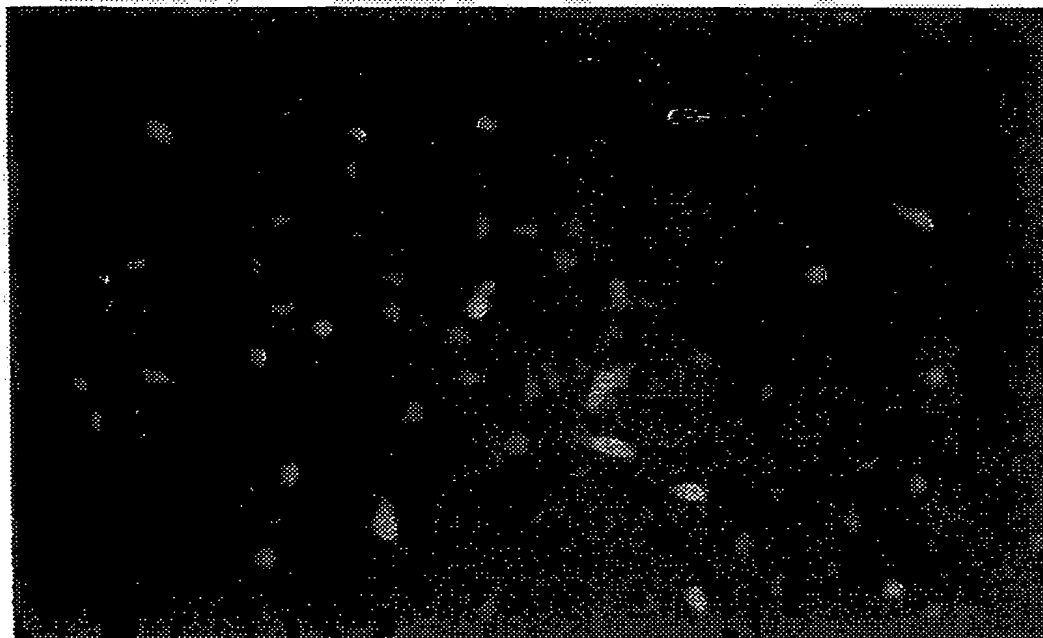


Fig 12 d

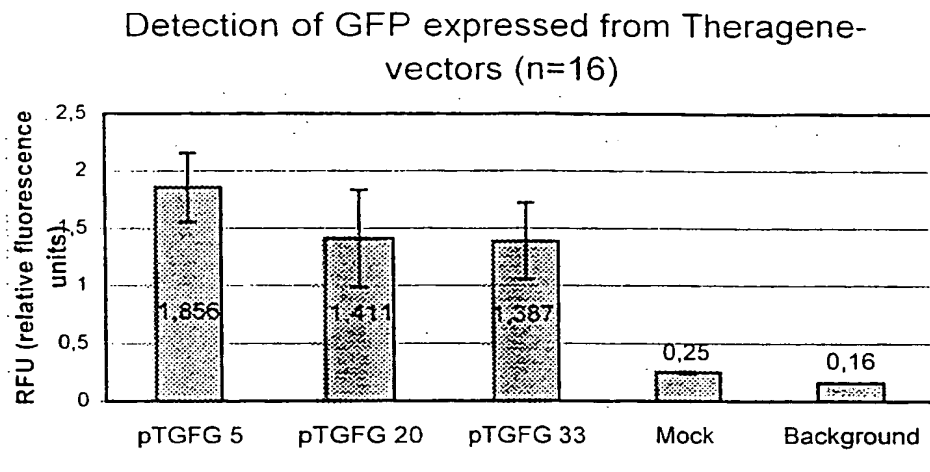
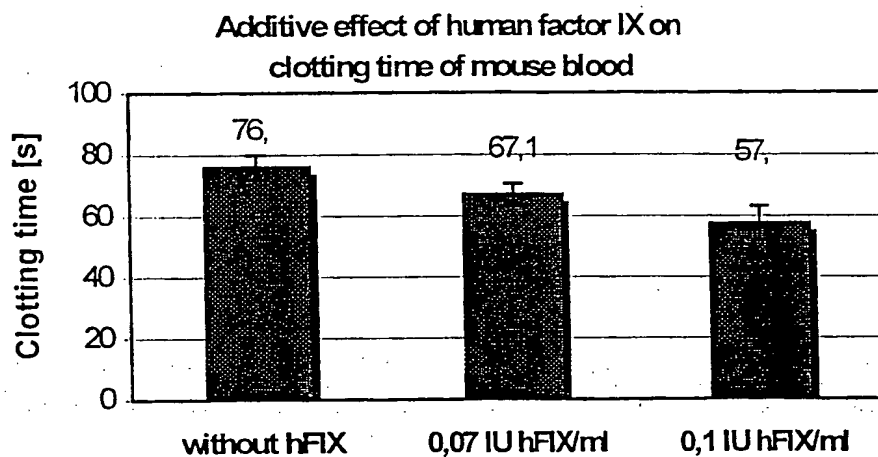
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Fig. 13

Fig. 14



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Fig. 15

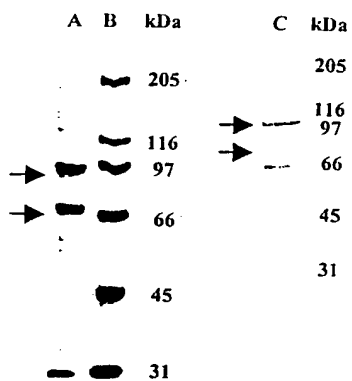
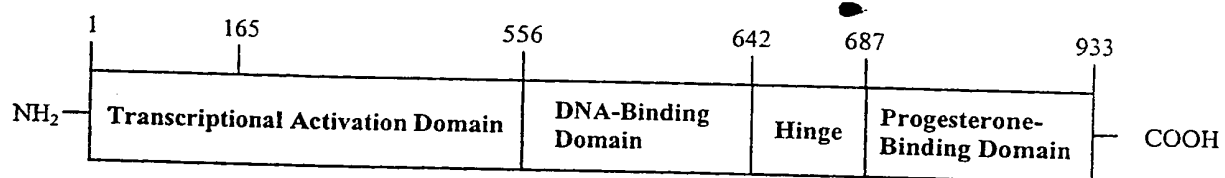




Fig. 16



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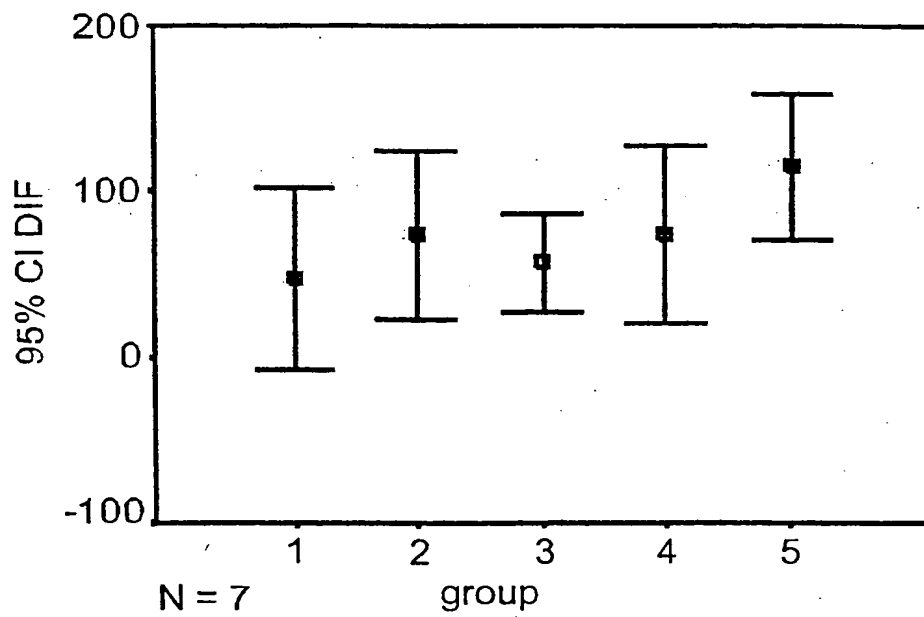


Fig. 17

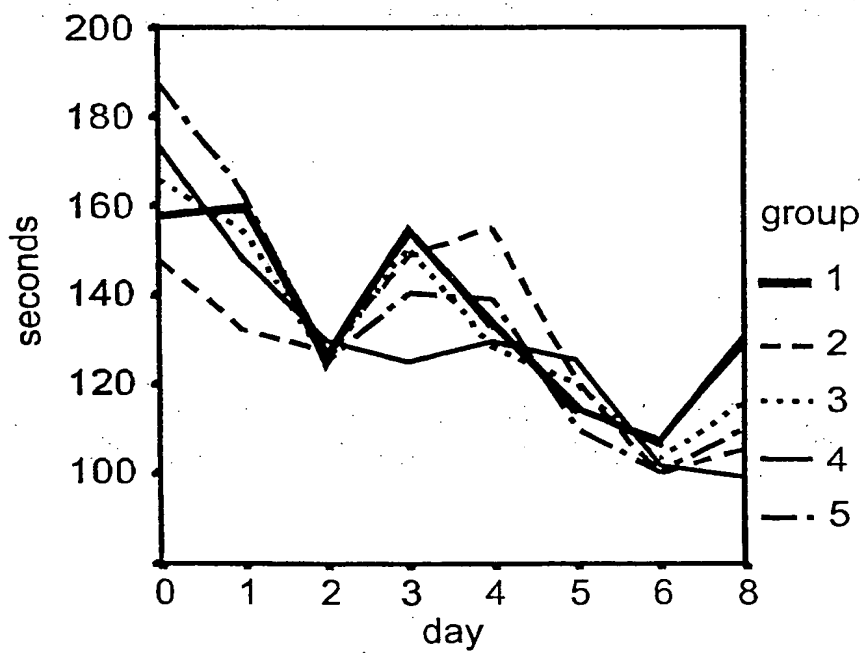
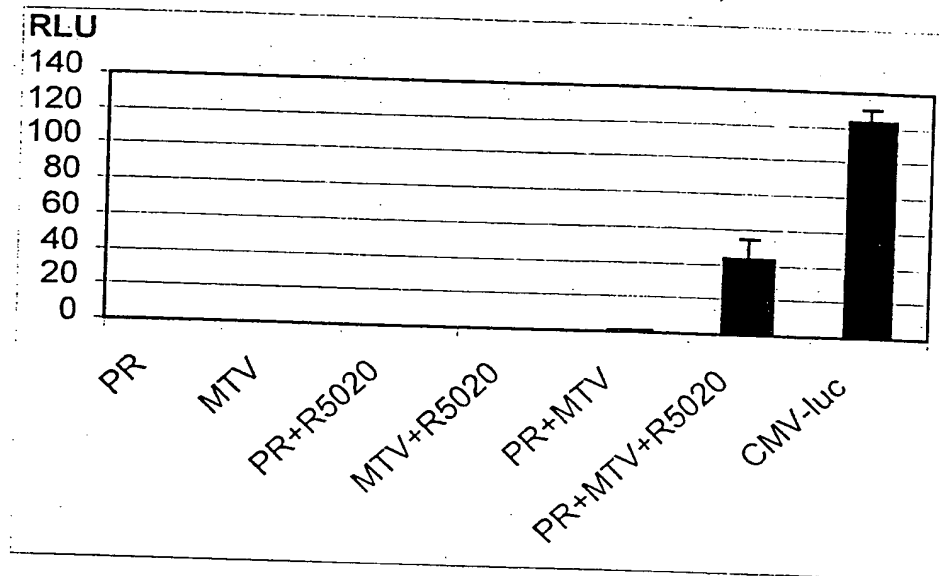


Fig. 18

Fig. 19



|     |             |            |            |            |            |             |
|-----|-------------|------------|------------|------------|------------|-------------|
| 1   | MTELKAKGPR  | APHVAGGPPS | PEVGSPLLCR | PAAGPFPGSQ | TSDTLPEVSA | IFISLDGLLF  |
| 61  | PRPCQGQDPS  | DEKTQDQOQL | SDVEGAYSRA | EATRGAGGSS | SSPPEKDSGL | LDSVLDTLLA  |
| 121 | PSGPGQSQPS  | PPACEVTSSW | CLFGPELPED | PPAAPATQRV | LSPLMSRSGC | KVGDSSGTAA  |
| 181 | AHKVLPRLGS  | PARQILLPAS | ESPHWSGAPV | KPSPQAAAVE | VEEEDGSESE | ESAGPLLKGG  |
| 241 | PRALGGAAAG  | GGAAAVPPGA | AAGGVALVPK | EDSRFSAPRV | ALVEQDAPMA | EGRSPLATTV  |
| 301 | MDFIHVPILP  | LNHALLAART | RQLEDESYD  | GGAGAASAF  | PPRSSPCASS | TPVAVGDFPD  |
| 361 | CAYPPDAEPK  | DDAYPLYSDF | QPPALKIKKE | EEGAEASARS | PRSYLVAGAN | PPAFPDFFPLG |
| 421 | PPPLPPPRAT  | PSRPGAAVT  | AAPASASVSS | ASSSGSTLEC | ILYKAEGAPP | QQGPFAPPPC  |
| 481 | KAPGASGCLL  | PRDGLPSTSA | SAAAAGAAPA | LYPALGLNGL | PQLGYQAAVL | KEGLPQVYPP  |
| 541 | YLNLYLRPDSE | ASQSPQYSFE | SLPQKICLIC | GDEASGCHYG | VLTCGSCVKF | FKRAMEGQHN  |
| 601 | YLCAGRNDIC  | VDKIRRNKCP | ACRLRKCCQA | GMVLGGRKFK | KFNKVRVVRA | LEDAVALPQL  |
| 661 | GVPNESQALS  | QRETFSPGQD | IQLIPPLINL | LMSIEPDVIY | AGHDNTKPD  | SSSLLTSLNQ  |
| 721 | LGERQLLSVV  | KWSKSLPGFR | NLHIDDQITL | IQYSWMSLMV | FGLGWSYKH  | VSGOMLYFAP  |
| 781 | DLILNEQRMK  | ESSFYSLCLT | MWQIPQEFVK | LQVSQEEFLC | MKVLLLLNTI | PLEGLRSQQT  |
| 841 | FEEMRSSYIR  | ELIKAIGLRQ | KGVVSSSQRF | YQLTKLLDNL | HDLVKQLHLY | CLNTFIQSRA  |
| 901 | LSVEFPMMMS  | EVIAAQLPKI | LAGMVKPLLF | HKK        |            |             |

Fig. 20

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1 ctgaccagcg cgcgcctccc cgcgcgcgcga cccaggaggt ggagatccct ccggtccagc  
61 cacattcaac acccactttc tcttccctct gccctatat tcccgaacc cctcctcct  
121 tcccttttcc ctctccctg gagacggggg aggagaaaag gggagtccag tcgtcatgac  
181 tgagctgaag gcaaagggtc cccgggctcc ccacgtggcg ggcggcccg cctccccga  
241 ggtcggatcc ccaactgctgt gtcgcccagc cgcagggtccg ttcccgggga gccagacctc  
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361 gccctgccag ggacaggacc cctccgacga aaagacgcag gaccagcagt cgtgtcggga  
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Fig. 21

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50 ctagagaacc cactgcttac tggcttatcg aaattaatac gactcactat agggagaccc 660  
aagcttgcac gccaatccg caaagggt atg cag cgc gtg aac atg atc atg 712  
Met Gln Arg Val Asn Met Ile Met  
55 1 5

gca gaa tca cca ggc ctc atc acc atc tgc ctt tta gga tat cta ctc 760  
Ala Glu Ser Pro Gly Leu Ile Thr Ile Cys Leu Leu Gly Tyr Leu Leu  
10 15 20

60

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|    | agt gct gaa tgt aca gtt ttt ctt gat cat gaa aac gcc aac aaa att | 808  |
|    | Ser Ala Glu Cys Thr Val Phe Leu Asp His Glu Asn Ala Asn Lys Ile |      |
|    | 25 30 35 40   |      |
| 5  | ctg aat cgg cca aag agg tat aat tca ggt aaa ttg gaa gag ttt gtt | 856  |
|    | Leu Asn Arg Pro Lys Arg Tyr Asn Ser Gly Lys Leu Glu Glu Phe Val |      |
|    | 45 50 55  |      |
| 10 | caa ggg aac ctt gag aga gaa tgt atg gaa gaa aag tgt agt ttt gaa | 904  |
|    | Gln Gly Asn Leu Glu Arg Glu Cys Met Glu Glu Lys Cys Ser Phe Glu |      |
|    | 60 65 70  |      |
| 15 | gaa gca cga gaa gtt ttt gaa aac act gaa aga aca act gaa ttt tgg | 952  |
|    | Glu Ala Arg Glu Val Phe Glu Asn Thr Glu Arg Thr Thr Glu Phe Trp |      |
|    | 75 80 85  |      |
| 20 | aag cag tat gtt gat gga gat cag tgt gag tcc aat cca tgt tta aat | 1000 |
|    | Lys Gln Tyr Val Asp Gly Asp Gln Cys Glu Ser Asn Pro Cys Leu Asn |      |
|    | 90 95 100   |      |
| 25 | ggc ggc agt tgc aag gat gac att aat tcc tat gaa tgt tgg tgt ccc | 1048 |
|    | Gly Gly Ser Cys Lys Asp Asp Ile Asn Ser Tyr Glu Cys Trp Cys Pro |      |
|    | 105 110 115 120   |      |
| 30 | ttt gga ttt gaa gga aag aac tgt gaa tta gat gta aca tgt aac att | 1096 |
|    | Phe Gly Phe Glu Gly Lys Asn Cys Glu Leu Asp Val Thr Cys Asn Ile |      |
|    | 125 130 135   |      |
| 35 | aag aat ggc aga tgc gag cag ttt tgt aaa aat agt gct gat aac aag | 1144 |
|    | Lys Asn Gly Arg Cys Glu Gln Phe Cys Lys Asn Ser Ala Asp Asn Lys |      |
|    | 140 145 150   |      |
| 40 | gtg gtt tgc tcc tgt act gag gga tat cga ctt gca gaa aac cag aag | 1192 |
|    | Val Val Cys Ser Cys Thr Glu Gly Tyr Arg Leu Ala Glu Asn Gln Lys |      |
|    | 155 160 165   |      |
| 45 | tcc tgt gaa cca gca gtg cca ttt cca tgt gga aga gtt tct gtt tca | 1240 |
|    | Ser Cys Glu Pro Ala Val Pro Phe Pro Cys Gly Arg Val Ser Val Ser |      |
|    | 170 175 180   |      |
| 50 | caa act tct aag ctc acc cgt gct gag act gtt ttt cct gat gtg gac | 1288 |
|    | Gln Thr Ser Lys Leu Thr Arg Ala Glu Thr Val Phe Pro Asp Val Asp |      |
|    | 185 190 195 200   |      |
| 55 | tat gta aat tct act gaa gct gaa acc att ttg gat aac atc act caa | 1336 |
|    | Tyr Val Asn Ser Thr Glu Ala Glu Thr Ile Leu Asp Asn Ile Thr Gln |      |
|    | 205 210 215   |      |
| 60 | agc acc caa tca ttt aat gac ttc act cgg gtt gtt ggt gga gaa gat | 1384 |
|    | Ser Thr Gln Ser Phe Asn Asp Phe Thr Arg Val Val Gly Gly Glu Asp |      |
|    | 220 225 230   |      |
| 65 | gcc aaa cca ggt caa ttc cct tgg cag gtt gtt ttg aat ggt aaa gtt | 1432 |
|    | Ala Lys Pro Gly Gln Phe Pro Trp Gln Val Val Leu Asn Gly Lys Val |      |
|    | 235 240 245   |      |
| 70 | gat gca ttc tgt gga ggc tct atc gtt aat gaa aaa tgg att gta act | 1480 |
|    | Asp Ala Phe Cys Gly Gly Ser Ile Val Asn Glu Lys Trp Ile Val Thr |      |
|    | 250 255 260   |      |

gct gcc cac tgt gtt gaa act ggt gtt aaa att aca gtt gtc gca ggt 1528  
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 265 270 275 280

5 gaa cat aat att gag gag aca gaa cat aca gag caa aag cga aat gtg 1576  
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 285 290 295

10 att cga att att cct cac cac aac tac aat gca gct att aat aag tac 1624  
 Ile Arg Ile Ile Pro His His Asn Tyr Asn Ala Ala Ile Asn Lys Tyr  
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15 aac cat gac att gcc ctt ctg gaa ctg gac gaa ccc tta gtg cta aac 1672  
 Asn His Asp Ile Ala Leu Leu Glu Leu Asp Glu Pro Leu Val Leu Asn  
 315 320 325

20 agc tac gtt aca cct att tgc att gct gac aag gaa tac acg aac atc 1720  
 Ser Tyr Val Thr Pro Ile Cys Ile Ala Asp Lys Glu Tyr Thr Asn Ile  
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ttc ctc aaa ttt gga tct ggc tat gta agt ggc tgg gga aga gtc ttc 1768  
 Phe Leu Lys Phe Gly Ser Gly Tyr Val Ser Gly Trp Gly Arg Val Phe  
 345 350 355 360

25 cac aaa ggg aga tca gct tta gtt ctt cag tac ctt aga gtt cca ctt 1816  
 His Lys Gly Arg Ser Ala Leu Val Leu Gln Tyr Leu Arg Val Pro Leu  
 365 370 375

30 gtt gac cga gcc aca tgt ctt cga tct aca aag ttc acc atc tat aac 1864  
 Val Asp Arg Ala Thr Cys Leu Arg Ser Thr Lys Phe Thr Ile Tyr Asn  
 380 385 390

35 aac atg ttc tgt gct ggc ttc cat gaa gga ggt aga gat tca tgt caa 1912  
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 395 400 405

40 gga gat agt ggg gga ccc cat gtt act gaa gtg gaa ggg acc agt ttc 1960  
 Gly Asp Ser Gly Gly Pro His Val Thr Glu Val Glu Gly Thr Ser Phe  
 410 415 420

tta act gga att att agc tgg ggt gaa gag tgt gca atg aaa ggc aaa 2008  
 Leu Thr Gly Ile Ile Ser Trp Gly Glu Glu Cys Ala Met Lys Gly Lys  
 425 430 435 440

45 tat gga ata tat acc aag gta tcc cgg tat gtc aac tgg att aag gaa 2056  
 Tyr Gly Ile Tyr Thr Lys Val Ser Arg Tyr Val Asn Trp Ile Lys Glu  
 445 450 455

50 aaa aca aag ctc act taatgggacg ggtcgagcgg ccgcgactct actagaggat 2111  
 Lys Thr Lys Leu Thr  
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&lt;211&gt; 461

&lt;212&gt; PRT

55 &lt;213&gt; Artificial Sequence

&lt;223&gt; Description of Artificial Sequence: vector pTGFG36

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 1 5 10 15

Ile Cys Leu Leu Gly Tyr Leu Leu Ser Ala Glu Cys Thr Val Phe Leu  
 20 25 30  
 5 Asp His Glu Asn Ala Asn Lys Ile Leu Asn Arg Pro Lys Arg Tyr Asn  
 35 40 45  
 Ser Gly Lys Leu Glu Glu Phe Val Gln Gly Asn Leu Glu Arg Glu Cys  
 50 55 60  
 10 Met Glu Glu Lys Cys Ser Phe Glu Glu Ala Arg Glu Val Phe Glu Asn  
 65 70 75 80  
 Thr Glu Arg Thr Thr Glu Phe Trp Lys Gln Tyr Val Asp Gly Asp Gln  
 85 90 95  
 15 Cys Glu Ser Asn Pro Cys Leu Asn Gly Gly Ser Cys Lys Asp Asp Ile  
 100 105 110  
 Asn Ser Tyr Glu Cys Trp Cys Pro Phe Gly Phe Glu Gly Lys Asn Cys  
 115 120 125  
 Glu Leu Asp Val Thr Cys Asn Ile Lys Asn Gly Arg Cys Glu Gln Phe  
 130 135 140  
 25 Cys Lys Asn Ser Ala Asp Asn Lys Val Val Cys Ser Cys Thr Glu Gly  
 145 150 155 160  
 Tyr Arg Leu Ala Glu Asn Gln Lys Ser Cys Glu Pro Ala Val Pro Phe  
 165 170 175  
 30 Pro Cys Gly Arg Val Ser Val Ser Gln Thr Ser Lys Leu Thr Arg Ala  
 180 185 190  
 35 Glu Thr Val Phe Pro Asp Val Asp Tyr Val Asn Ser Thr Glu Ala Glu  
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 Thr Ile Leu Asp Asn Ile Thr Gln Ser Thr Gln Ser Phe Asn Asp Phe  
 210 215 220  
 40 Thr Arg Val Val Gly Gly Glu Asp Ala Lys Pro Gly Gln Phe Pro Trp  
 225 230 235 240  
 Gln Val Val Leu Asn Gly Lys Val Asp Ala Phe Cys Gly Gly Ser Ile  
 245 250 255  
 45 Val Asn Glu Lys Trp Ile Val Thr Ala Ala His Cys Val Glu Thr Gly  
 260 265 270  
 Val Lys Ile Thr Val Val Ala Gly Glu His Asn Ile Glu Glu Thr Glu  
 275 280 285  
 50 His Thr Glu Gln Lys Arg Asn Val Ile Arg Ile Ile Pro His His Asn  
 290 295 300  
 55 Tyr Asn Ala Ala Ile Asn Lys Tyr Asn His Asp Ile Ala Leu Leu Glu  
 305 310 315 320  
 Leu Asp Glu Pro Leu Val Leu Asn Ser Tyr Val Thr Pro Ile Cys Ile  
 325 330 335  
 60 Ala Asp Lys Glu Tyr Thr Asn Ile Phe Leu Lys Phe Gly Ser Gly Tyr  
 340 345 350

Val Ser Gly Trp Gly Arg Val Phe His Lys Gly Arg Ser Ala Leu Val  
                   355                                  360                                  365

5 Leu Gln Tyr Leu Arg Val Pro Leu Val Asp Arg Ala Thr Cys Leu Arg  
                   370                                  375                                  380

Ser Thr Lys Phe Thr Ile Tyr Asn Asn Met Phe Cys Ala Gly Phe His  
 385                                  390                                  395                                  400

10 Glu Gly Gly Arg Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro His Val  
                                   405                                  410                                  415

15 Thr Glu Val Glu Gly Thr Ser Phe Leu Thr Gly Ile Ile Ser Trp Gly  
                                   420                                  425                                  430

Glu Glu Cys Ala Met Lys Gly Lys Tyr Gly Ile Tyr Thr Lys Val Ser  
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10 <210> 8  
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15 <400> 8  
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20 <210> 9  
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25 <400> 9  
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30 <210> 10  
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 <212> DNA  
 <213> Homo sapiens

35 <400> 10  
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40 <210> 11  
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45 <400> 11  
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50 <210> 12  
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60 <220>  
 <223> Description of Artificial Sequence: vector pTGFG53

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|    | atagcccata | tatggagttc | cgcgttacat  | aacttacggt  | aaatggcccg  | cctggctgac  | 120  |
|    | cgcccacga  | cccccgcca  | ttgacgtcaa  | taatgacgta  | tggtcccata  | gtaacgccaa  | 180  |
| 5  | tagggacttt | ccattgacgt | caatgggtgg  | agtattttacg | gtaaactgcc  | cacttggcag  | 240  |
|    | tacatcaagt | gtatcatatg | ccaagtacgc  | cccctattga  | cgtcaatgac  | ggtaaatggc  | 300  |
|    | ccgcctggca | ttatgcccag | tacatgacct  | tatgggactt  | tcctacttgg  | cagtacatct  | 360  |
|    | acgtattagt | catcgctatt | accatgggtga | tgcggttttg  | gcagtacatc  | aatgggcgtg  | 420  |
|    | gatagcgggt | tgactcacgg | ggatttccaa  | gtctccaccc  | cattgacgtc  | aatgggagtt  | 480  |
| 10 | tgttttggca | ccaaaatcaa | cgggactttc  | caaaatgtcg  | taacaactcc  | gccccattga  | 540  |
|    | cgcaaattgg | cggtaggcgt | gtacgggtgg  | aggtctatat  | aagcagagct  | ctctggctaa  | 600  |
|    | ctagagaacc | cactgcttac | tggcttatcg  | aaattaatac  | gactcactat  | agggagacc   | 660  |
|    | aagcttgcat | gccaattccg | caaagggtat  | gcagcgcgtg  | aacatgatca  | tggcagaatc  | 720  |
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| 15 | tcttgatcat | gaaaacgcca | acaaaattct  | gaatcggcca  | aagagggtata | attcaggtaa  | 840  |
|    | attggaagag | ttgtttcaag | ggaaccttga  | gagagaatgt  | atggaagaaa  | agtgtagttt  | 900  |
|    | tgaagaagca | cgagaagtgt | ttgaaaacac  | tgaagaagca  | actgaatttt  | ggaagcagta  | 960  |
|    | tgttgatgga | gatcagtggt | agtccaatcc  | atgttttaaat | ggcggcagtt  | ggaaagagta  | 1020 |
| 20 | cattaattcc | tatgaatgtt | ggtgtccctt  | tggatttgaa  | ggaaagaact  | gtgaattaga  | 1080 |
|    | tgtaacatgt | aacattaaga | atggcagatg  | cgagcagttt  | tgtaaaaata  | gtgctgataa  | 1140 |
|    | caaggtgggt | tgctcctgta | ctgagggata  | tcgacttgca  | gaaaaccaga  | agtcctgtga  | 1200 |
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|    | ggataacatc | actcaaagca | cccaatcatt  | taatgacttc  | actcgggttg  | ttggtggaga  | 1380 |
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|    | tggtgttaaa | attacagttg | tcgcaggtga  | acataatatt  | gaggagacag  | aacatacaga  | 1560 |
|    | gcaaaaagca | aatgtgattc | gaattattcc  | taccacaac   | tacaatgcag  | ctattaataa  | 1620 |
| 30 | gtacaaccat | gacattgccc | ttctgggaact | ggacgaaccc  | ttagtgtctaa | acagctacgt  | 1680 |
|    | tacacctatt | tgcattgctg | acaaggaata  | cacgaacatc  | ttcctcaaat  | ttggatctgg  | 1740 |
|    | ctatgtaagt | ggctggggaa | gagtcctcca  | caaagggaga  | tcagctttag  | ttcttcagta  | 1800 |
|    | ccttagagtt | ccacttggtg | accgagccac  | atgtcttcga  | tctacaaagt  | tcaccatcta  | 1860 |
|    | taacaacatg | ttctgtgctg | gcttccatga  | aggaggtaga  | gattcatgtc  | aaggagatag  | 1920 |
|    | tgggggaccc | catgttactg | aagtggaaag  | gaccagtttc  | ttacttgga   | ttattagctg  | 1980 |
| 35 | gggtgaagag | tgtgcaatga | aaggcaata   | tggaatatat  | accaaggtat  | ccgggtatgt  | 2040 |
|    | caactggatt | aaggaaaaaa | caaagctcac  | ttaatgggat  | cggctcgagcg | gccgcgactc  | 2100 |
|    | tactagagga | tctttgtgaa | ggaaccttac  | ttctgtgggt  | tgacataatt  | ggacaaacta  | 2160 |
|    | cctacagaga | tttaaagctc | taaggtaaat  | ataaaatttt  | taagtgtata  | atgtgttaaa  | 2220 |
|    | ctactgattc | taattgtttg | tgtatttttag | attccaacct  | atggaactga  | tgaatgggag  | 2280 |
| 40 | cagtgtggga | atgcctttaa | tgaggaaaaa  | ctgttttctg  | cagaagaaat  | gccatctagt  | 2340 |
|    | gatgatgagg | ctactgctga | ctctcaacat  | tctactcctc  | caaaaaagaa  | gagaaaggtg  | 2400 |
|    | gaagacccca | aggactttcc | ttcagaattg  | ctaagttttt  | tgagtcatgc  | tggtgttagt  | 2460 |
|    | aatagaactc | ttgcttgctt | tgctattttac | accacaaagg  | aaaaagctgc  | actgctatac  | 2520 |
|    | aagaaaatta | tggaaaaata | ttctgtaacc  | tttataagta  | ggcataacag  | ttataatcat  | 2580 |
| 45 | aacatactgt | tttttcttac | tccacacagg  | catagagtgt  | ctgctattaa  | taactatgct  | 2640 |
|    | caaaaattgt | gtacctttag | ctttttaatt  | tgtaaaaggg  | tttaataagg  | atattttgatg | 2700 |
|    | tatagtgcct | tgactagaga | tcataatcag  | ccataccaca  | tttgtagagg  | ttttacttgc  | 2760 |
|    | tttaaaaaac | ctccacacac | tccccctgaa  | cctgaaacat  | aaaatgaatg  | caattgttgt  | 2820 |
|    | tgtttaactg | tttattgcag | cttataatgg  | ttacaaataa  | agcaatagca  | tcacaaattt  | 2880 |
| 50 | cacaaataaa | gcattttttt | caactgcattc | tagttgtggt  | ttgtccaaac  | tcataaatgt  | 2940 |
|    | atcttatcat | gtctggatcc | ccggggggta  | ccagcttcgt  | agctagaaca  | tcattgttctg | 3000 |
|    | ggatatcagc | ttcgtagcta | gaacatcatg  | ttctgggtacc | cccgtcttag  | agcgaattaa  | 3060 |
|    | ttcactggcc | gtcgttttac | aacgtcgtga  | ctgggaaaac  | cctggcggtta | cccaacttaa  | 3120 |
|    | tcgccttgca | gcacatcccc | ctttcgccag  | ctggcgtaat  | agcgaagagg  | cccgaccoga  | 3180 |
| 55 | tcgcctttcc | caacagttgc | gcagcctgaa  | tggcgaatgg  | cgctgatgc   | ggtattttct  | 3240 |
|    | ccttacgcat | ctgtgcggtg | tttcacaccg  | catatggtgc  | actctcagta  | caatctgctc  | 3300 |
|    | tgatgccgca | tagttaagcc | agccccgaca  | cccgcgaaca  | cccgtctgacg | cgccctgacg  | 3360 |
|    | ggcttgtctg | ctcccgcat  | ccgcttagac  | acaagctgtg  | accgtctccg  | ggagctgcag  | 3420 |
|    | gtgtcagagg | ttttcacccg | catcacccga  | acgcgcgaga  | cgaaagggcg  | gggtaccaga  | 3480 |
| 60 | acatgatgtt | ctagctacga | agctgatatc  | ccagaacatg  | atgttctagc  | tacgaagctg  | 3540 |
|    | gtaccccggc | ctcgtgatac | gcctattttt  | ataggttaat  | gtcatgataa  | taatggtttc  | 3600 |
|    | ttagacgtca | ggtggcactt | ttcggggaaa  | tgtgcgcgga  | acccttattt  | gtttattttt  | 3660 |

|    |             |             |            |             |            |             |      |
|----|-------------|-------------|------------|-------------|------------|-------------|------|
|    | ctaaatacat  | tcaaataatgt | atccgctcat | gagacaataa  | ccctgataaa | tgcttcaata  | 3720 |
|    | atattgaaaa  | aggaagagta  | tgagtattca | acattttccgt | gtcgccctta | ttcccttttt  | 3780 |
|    | tgccggcattt | tgcccttcctg | tttttgctca | cccagaaaacg | ctggtgaaag | taaaagatgc  | 3840 |
|    | tgaagatcag  | ttgggtgcac  | gagtgggtta | catcgaaactg | gatctcaaca | gcggtaagat  | 3900 |
| 5  | ccttgagagt  | tttcgccccg  | aagaacgttt | tccaatgatg  | agcactttta | aagttctgct  | 3960 |
|    | atgtggcgcg  | gtattatccc  | gtattgacgc | cgggcaagag  | caactcggtc | gccgcataca  | 4020 |
|    | ctattctcag  | aatgacttgg  | ttgagtactc | accagtcaca  | gaaaagcatc | ttacggatgg  | 4080 |
|    | catgacagta  | agagaattat  | gcagtgtctg | cataaccatg  | agtataaca  | ctgcggccaa  | 4140 |
|    | cttacttctg  | acaacgatcg  | gaggaccgaa | ggagctaacc  | gcttttttgc | acaacatggg  | 4200 |
| 10 | ggatcatgta  | actcgcccttg | atcggtggga | accggagctg  | aatgaagcca | taccaaacga  | 4260 |
|    | cgagcgtgac  | accacgatgc  | ctgtagcaat | ggcaacaacg  | ttgcgcaaac | tattaactgg  | 4320 |
|    | cgaactactt  | actctagctt  | cccggcaaca | attaatagac  | tggtatggag | cggataaagt  | 4380 |
|    | tgcaggacca  | cttctgcgct  | cgcccttcc  | ggctggctgg  | tttattgctg | ataaatctgg  | 4440 |
|    | agccggtgag  | cgtgggtctc  | gcggtatcat | tgacgactg   | gggccagatg | gtaagccctc  | 4500 |
| 15 | ccgtatcgta  | gttatctaca  | cgacggggag | tcaggcaact  | atggatgaac | gaaatagaca  | 4560 |
|    | gatcgctgag  | ataggtgcct  | cactgattaa | gcattggtaa  | ctgtcagacc | aagtttactc  | 4620 |
|    | atatactagt  | tagattgatt  | taaaacttca | tttttaattt  | aaaaggatct | aggtgaagat  | 4680 |
|    | cctttttgat  | aatctcatga  | ccaaaatccc | tttaacgtgag | ttttcgcttc | actgagcgtc  | 4740 |
|    | agaccccgta  | gaaaagatca  | aaggatcttc | ttgagatcct  | ttttttctgc | gcgtaactgt  | 4800 |
| 20 | ctgcttgcaa  | acaaaaaac   | caccgctacc | agcggtggtt  | tgtttgccgg | atcaagagct  | 4860 |
|    | accaactctt  | tttccgaagg  | taactggctt | cagcagagcg  | cagataccaa | atactgtcct  | 4920 |
|    | tctagttag   | ccgtagttag  | gccaccactt | caagaactct  | gtagcaccgc | ctacatacct  | 4980 |
|    | cgctctgcta  | atcctgttac  | cagtggctgc | tgccagtggc  | gataagtcgt | gtcttaccgg  | 5040 |
|    | gttggactca  | agacgatagt  | tacggataag | gcgcagcgg   | cgggctgaac | gggggggttcg | 5100 |
| 25 | tgcacacagc  | ccagcttggg  | gcgaacgacc | tacaccgaac  | tgagatacct | acagcgtgag  | 5160 |
|    | ctatgagaaa  | gcgccacgct  | tcccgaagg  | agaaaggcgg  | acaggtatcc | ggtaagcggc  | 5220 |
|    | agggtcggaa  | caggagagcg  | cacgagggag | cttccagggg  | gaaacgcctg | gtatctttat  | 5280 |
|    | agtcctgtcg  | ggtttcgcca  | cctctgactt | gagcgtcgat  | ttttgtgatg | ctcgtcagg   | 5340 |
|    | gggcggagcc  | tatggaaaaa  | cgccagcaac | gcgccctttt  | tacggttcc  | ggccttttgc  | 5400 |
| 30 | tggccttttg  | ctcacatgtt  | ctttcctgcg | ttatccccg   | attctgtgga | taaccgtatt  | 5460 |
|    | accgcctttg  | agttagctga  | taccgctcgc | cgcagccgaa  | cgaccgagcg | cagcagatca  | 5520 |
|    | gtgagcgagg  | aagcggaaga  | gcgcccaata | cgaaaccgc   | ctctccccgc | gcgttggccg  | 5580 |
|    | attcattaat  | gcagctggca  | cgacaggttt | cccagactgga | aagcgggcag | tgagcgcaac  | 5640 |
|    | gcaattaatg  | tgagttagct  | cactcattag | gcaccccagg  | ctttacactt | tatgcttccg  | 5700 |
| 35 | gctcgtatgt  | tgtgtggaat  | tgtgagcgga | taacaatttc  | acacaggaaa | cagctatgac  | 5760 |
|    | catgattacg  | ccaagctctc  | tagagctcta | gagctctaga  | gctctagaga | gcttgcatgc  | 5820 |
|    | cggggtacca  | gcttcgtagc  | tagaacatca | tgttctggga  | tatcagcttc | gtagctagaa  | 5880 |
|    | catcatgttc  | tggtaccccg  | gtcga      |             |            |             | 5905 |

&lt;210&gt; 14

&lt;211&gt; 6052

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: vector pTGFG64

&lt;400&gt; 14

|    |             |            |            |             |             |             |     |
|----|-------------|------------|------------|-------------|-------------|-------------|-----|
| 50 | cgcggttgaca | ttgattattg | actagttatt | aatagtaatc  | aattacgggg  | tcattagttc  | 60  |
|    | atagcccata  | tatggagttc | cgcgttacat | aacttacggg  | aaatggcccg  | cctggctgac  | 120 |
|    | cgcccaacga  | ccccgcgcca | ttgacgtcaa | taatgacgta  | tgttcccata  | gtaacgcca   | 180 |
|    | tagggacttt  | ccattgacgt | caatgggtgg | agtattttacg | gtaaaactgcc | cacttggcag  | 240 |
| 55 | tacatcaagt  | gtatcatatg | ccaagtacgc | cccctattga  | cgtcaatgac  | ggtaaattggc | 300 |
|    | ccgcctggca  | ttatgccag  | tacatgacct | tatgggactt  | tcctacttgg  | cagtacatct  | 360 |
|    | acgtattagt  | catcgctatt | accatgggtg | tgcggttttg  | gcagtacatc  | aatgggcgtg  | 420 |
|    | gatagcgggt  | tgactcacgg | ggatttccaa | gtctccaccc  | cattgacgtc  | aatgggagtt  | 480 |
|    | tgttttggca  | ccaaaatcaa | cgggactttc | caaaatgtcg  | taacaactcc  | gccccattga  | 540 |
|    | cgcaaatggg  | cggtaggcgt | gtacgggtgg | aggtctatat  | aagcagagct  | ctctggctaa  | 600 |
| 60 | ctagagaacc  | cactgcttac | tggttatcgc | aaattaatac  | gactcactat  | aggagaccc   | 660 |
|    | aagcttgcac  | gccaatccg  | caaaggttat | gcagcgcgtg  | aacatgatca  | tgccagaatc  | 720 |
|    | accaggcttc  | atcaccatct | gccttttagg | atatctactc  | agtgtgtaat  | gtacagtttt  | 780 |

|    |             |             |             |             |             |             |      |
|----|-------------|-------------|-------------|-------------|-------------|-------------|------|
|    | tcttgatcat  | gaaaacgcca  | acaaaattct  | gaatcggcca  | aagagggtata | attcagggtaa | 840  |
|    | attggaagag  | tttgttcaag  | ggaaccttga  | gagagaatgt  | atggaagaaa  | agtgtagttt  | 900  |
|    | tgaagaagca  | cgagaagttt  | ttgaaaacac  | tgaagaagca  | actgaatttt  | ggaagcagta  | 960  |
|    | tgttgatgga  | gatcagtggt  | agtccaatcc  | atgttttaaat | ggcggcagtt  | gcaaggatga  | 1020 |
| 5  | cattaattcc  | tatgaatggt  | ggtgtccctt  | tggatttgaa  | ggaaagaact  | gtgaattaga  | 1080 |
|    | tgtaacatgt  | aacattaaga  | atggcagatg  | cgagcagttt  | tgtaaaaata  | gtgctgataa  | 1140 |
|    | caagggtggt  | tgctcctgta  | ctgagggata  | tcgacttgca  | gaaaaccaga  | agtctgtgta  | 1200 |
|    | accagcagtg  | ccatttccat  | gtggaagagt  | ttctgtttca  | caaacttcta  | agctcaccgc  | 1260 |
|    | tgctgagact  | gtttttcctg  | atgtggacta  | tgtaaatctt  | actgaagctg  | aaaccatttt  | 1320 |
| 10 | ggataaacatc | actcaaagca  | cccaatcatt  | taatgacttc  | actcgggttg  | ttggtggaga  | 1380 |
|    | agatgccaaa  | ccagggtcaat | tcccttggca  | ggttgttttg  | aatggtaaa   | ttgatgcatt  | 1440 |
|    | ctgtggaggc  | tctatcgtaa  | atgaaaaatg  | gattgttaact | gctgcccact  | gtgttgaaac  | 1500 |
|    | tggtgttaaaa | attacagttg  | tcgcaggtga  | acataatatt  | gaggagacag  | aacatacaga  | 1560 |
|    | gcaaaaagcga | aatgtgattc  | gaattattcc  | tcaccacaac  | tacaatgcag  | ctattaataa  | 1620 |
| 15 | gtacaacatc  | gacattgccc  | ttctggaact  | ggacgaaccc  | ttagtgtctaa | acagctacgt  | 1680 |
|    | tacacctatt  | tgcatgtctg  | acaagggaata | cacgaacatc  | ttcctcaaat  | ttggatctgg  | 1740 |
|    | ctatgtaagt  | ggctggggaa  | gagtcctcca  | caaagggaga  | tcagctttag  | ttcttcagta  | 1800 |
|    | ccttagagtt  | ccacttggtg  | accgagccac  | atgtcttcga  | tctacaaagt  | tcaccatcta  | 1860 |
|    | taacaacatg  | ttctgtgctg  | gcttccatga  | aggaggtaga  | gattcatgtc  | aaggagatag  | 1920 |
| 20 | tggggggaccc | catgttactg  | aagtgggaagg | gaccagtttc  | ttactggaa   | ttattagctg  | 1980 |
|    | gggtgaagag  | tgtgcaatga  | aaggcaataa  | tggaaatat   | accaaggtat  | cccggtatgt  | 2040 |
|    | caactggatt  | aaggaaaaaa  | caaagctcac  | ttaatgggat  | cggtcgagcg  | gccgcgactc  | 2100 |
|    | tactagagga  | tctttgtgaa  | ggaaccttac  | ttctgtgggt  | tgacataaatt | ggacaaacta  | 2160 |
|    | cctacagaga  | tttaaagctc  | taaggtaaat  | ataaaatttt  | taagtgtata  | atgtgttaaa  | 2220 |
| 25 | ctactgattc  | taattgtttg  | tgtatttttag | attccaacct  | atggaactga  | tgaatgggag  | 2280 |
|    | cagtgggtgga | atgcctttaa  | tgaggaaaac  | ctgttttgct  | cagaagaaat  | gccatctagt  | 2340 |
|    | gatgatgagg  | ctactgctga  | ctctcaacat  | tctactcttc  | caaaaaagaa  | gagaaaggta  | 2400 |
|    | gaagacccca  | aggactttcc  | ttcagaattg  | ctaagttttt  | tgagtcatgc  | tgtgttttagt | 2460 |
| 30 | aatagaactc  | ttgcttgctt  | tgctattttac | accacaaagg  | aaaaagctgc  | actgctatac  | 2520 |
|    | aagaaaatta  | tggaaaaata  | ttctgtaacc  | tttataagta  | ggcataacag  | ttataatcat  | 2580 |
|    | aacatactgt  | tttttcttac  | tcacacacag  | catagagtgt  | ctgctattaa  | taactatgct  | 2640 |
|    | caaaaattag  | gtacctttag  | cttttttaatt | tgtaaagggg  | ttataaagga  | atattttgatg | 2700 |
|    | tatagtgcct  | tgactagaga  | tcataatcac  | ccataaccaca | tttgtagagg  | ttttacttgc  | 2760 |
| 35 | tttaaaaaaac | ctccacacac  | tccccctgaa  | cctgaaacat  | aaaatgaatg  | caattgttgt  | 2820 |
|    | tgtaaacttg  | tttattgcag  | cttataatgg  | ttacaaataa  | agcaatagca  | tcacaaaattt | 2880 |
|    | cacaaataaa  | gcattttttt  | cactgcattc  | tagttgtggt  | ttgtccaaac  | tcatcaatgt  | 2940 |
|    | atcttatcat  | gtctggatcc  | ccgggggggta | ccagcttcgt  | agctagaaca  | tcatgttctg  | 3000 |
|    | ggatagcage  | ttcgtagcta  | gaacatcatg  | ttctggtacc  | cccctctaga  | gcgaattaat  | 3060 |
| 40 | tcaactggcg  | tcgctttaca  | acgtcgtgac  | tgggaaaacc  | ctggcggtac  | ccaacttaat  | 3120 |
|    | cgcccttgcag | cacatccccc  | tttcgccagc  | tggcgtaata  | gcgaagaggc  | ccgcacccat  | 3180 |
|    | cgcccttccc  | aacagttgcg  | cagcctgaat  | ggcgaaatggc | ggggtaaccag | cttcgtagct  | 3240 |
|    | agaacatcat  | gttctgggat  | atcagcttcg  | tagctagaac  | atcatgttct  | ggtaccccg   | 3300 |
|    | ctgatgcggg  | atcttctcct  | tacgcattctg | tgcggtattt  | cacaccgcat  | atggtgcact  | 3360 |
|    | ctcagtacaa  | tctgctctga  | tgcgcgatag  | ttaaagccagc | cccgcacccc  | gccaacaccc  | 3420 |
| 45 | gctgacgcgc  | cctgacgggc  | ttgtctgctc  | ccgctatccg  | cttacagaca  | agctgtgacc  | 3480 |
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|    | aagggcacca  | gaacatgatg  | ttctagctac  | gaagctgata  | tcccagaaca  | tgatgttcta  | 3600 |
|    | gctacgaagc  | tggtaacccg  | cctcgtgata  | cgcctatttt  | tatagggttaa | tgtcatgata  | 3660 |
|    | ataatgggtt  | cttagacgtc  | aggtggcact  | tttcggggaa  | atgtgcgcgg  | aacccttatt  | 3720 |
| 50 | tgttttatttt | tctaaaataa  | ttcaaatatg  | tatccgctca  | tgagacaata  | accctgataa  | 3780 |
|    | atgcttcaat  | aatattgaaa  | aaggaagagt  | gatgatttc   | aacatttccg  | tgtcgccctt  | 3840 |
|    | attccctttt  | ttgcggcatt  | ttgccttcc   | gtttttgtct  | acccagaaac  | gctggtgaaa  | 3900 |
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|    | agcggtaaga  | tccttgagag  | ttttcgcccc  | gaagaacggt  | ttccaatgat  | gagcactttt  | 4020 |
| 55 | aaagtctctg  | tatgtggcgc  | ggtattatcc  | cgtattgacg  | ccgggcaaga  | gcaactcggt  | 4080 |
|    | cgccgcatac  | actattctca  | gaatgacttg  | gttgagtact  | caccagtcc   | agaaaagcat  | 4140 |
|    | cttacggatg  | gcatgacagt  | aagagaatta  | tgagctgctg  | ccataacccat | gagtgtatac  | 4200 |
|    | actgcggcca  | acttacttct  | gacaacgatc  | ggaggaccga  | aggagctaac  | cgcttttttg  | 4260 |
|    | cacaacatgg  | gggatcatgt  | aactcgcctt  | gatcgttggg  | aaccggagct  | gaatgaagcc  | 4320 |
| 60 | ataccaaacg  | acgagcgtga  | caccacgatg  | cctgtagcaa  | tggcaacaac  | gttgcgcaaa  | 4380 |
|    | ctattaactg  | gcgaactact  | tactctagct  | tcccggcaac  | aattaataga  | ctggatggag  | 4440 |
|    | gcggataaag  | ttgcaggacc  | acttctgcgc  | tccgcccctt  | cggctggctg  | gtttattgct  | 4500 |



5 gataaatctg gagccggtga gcggtgggtct cgcggtatca ttgcagcact ggggccagat 4560  
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 20 cccagaacat gatgttctag ctacgaagct ggtaccccag cggaagagcg cccaatacgc 5700  
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 gactggaaag cgggcagtga gcgcaacgca attaattgta gttagctcac tcattaggca 5820  
 ccccaggctt tacactttat gcttccggct cgtatgttgt gtggaattgt gagcggataa 5880  
 caatttcaca caggaaacag ctatgaccat gattacgcca agctctctag agctctagag 5940  
 25 ctctagagct cttagagagct tgcattgccg ggtaccagct tcgtagctag aacatcatgt 6000  
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30 <210> 15  
 <211> 4344  
 <212> DNA  
 <213> Artificial Sequence

35 <220>  
 <223> Description of Artificial Sequence: vector pTGFG67

40 <400> 15  
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 cgcccaacga ccccgccca ttgacgtcaa taatgacgta tgttcccata gtaacgccaa 180  
 tagggacttt ccattgacgt caatgggtgg agtatttacg gtaaactgcc cacttggcag 240  
 tacatcaagt gtatcatatg ccaagtacgc cccctattga cgtcaatgac ggtaaattggc 300  
 cgccctggca ttatgccagc tacatgacct tatgggactt tccctacttg cagtacatct 360  
 45 acgtattagt catcgctatt accatgggtga tgcggttttg gcagtlacatc aatgggcgtg 420  
 gatagcgggt tgactcacgg ggatttccaa gtctccaccc cattgacgtc aatgggagtt 480  
 tgttttggca ccaaaatcaa cgggactttc caaaatgtcg taacaactcc gccccattga 540  
 cgcaaattgg cggtaggcgt gtacgggtggg aggtctatat aagcagagct ctctggctaa 600  
 cttagaagcc cactgcttac tggctttatc aaattaatac gactcactat agggagaccc 660  
 50 aagcttgacc tcgagcaagc ggccgcgact ctactagagg atctttgtga aggaacctta 720  
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 tataaaattt ttaagtgtat aatgtgttaa actactgatt ctaattgttt gtgtatttta 840  
 gattccaacc tatggaactg atgaatggga gcagtgtgtg aatgccttta atgaggaaaa 900  
 cctgttttgc tcagaagaaa tgccatctag tgatgatgag gctactgctg actctcaaca 960  
 55 ttctactcct ccaaaaaaga agagaaaggt agaagacccc aaggactttc cttcagaatt 1020  
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 gcatagagtg tctgtatta ataactatgc tcaaaaattg tgtaccttta gctttttaat 1260  
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5 ctagtgtgtgg tttgtccaaa ctcatcaatg tatcttatca tgtctggatc cccgggtacc 1560  
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 15 gaacatcatg ttctgggtacc cccctcgtga tacgcctatt tttataggtt aatgtcatga 2100  
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 30 ctgataaatc tggagccggg gagcgtgggt ctgcggtat cctcactgat taagcattgg taactgtcag 3120  
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 35 tctaggtgaa gtcagacccc ctgtgtcttg caaacaacaa aaccaccgct accagcgtg gtttgtttgc 3360  
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 tggataaccg tattaccgcc tttgagttag ctgataccgc tcgccgcagc cgaacgaccg 4020  
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 50 ccgcgcgttg gccgattcat taatgcagct ggcacgacag gtttcccagc tggaaaagcg 4140  
 gcagtgagcg caacgcaatt aatgtgagtt agctcactca ttaggcaccc caggctttac 4200  
 actttatgct tccggctcgt atgttgtgtg gaattgtgag cggataacaa tttcacacag 4260  
 gaaacagcta tgaccatgat tacgccaagc tctctagagc tctagagctc tagagctcta 4320  
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50 <210> 16  
 <211> 4496  
 <212> DNA  
 <213> Artificial Sequence

55 <220>  
 <223> Description of Artificial Sequence: vector pTGFG82

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 atagccata tatggagttc cgcgttacat aacttacggt aaatggcccg cctggctgac 120  
 cgcccaacga ccccgcccca ttgacgtcaa taatgacgta tgttcccata gtaacgccaa 180

|    |             |             |             |             |             |             |      |
|----|-------------|-------------|-------------|-------------|-------------|-------------|------|
|    | tagggacttt  | ccattgacgt  | caatgggtgg  | agtattttacg | gtaaactgcc  | cacttggcag  | 240  |
|    | tacatcaagt  | gtatcatatg  | ccaagtacgc  | cccctattga  | cgtcaatgac  | ggtaaattggc | 300  |
|    | ccgcctggca  | ttatgcccgag | tacatgacct  | tatgggactt  | tcctacttgg  | cagtacatct  | 360  |
| 5  | acgtattagt  | catcgctatt  | accatgggtga | tgcggttttg  | gcagtacatc  | aatgggcgtg  | 420  |
|    | gatagcgggt  | tgactcacgg  | ggatttccaa  | gtctccaccc  | cattgacgtc  | aatgggagtt  | 480  |
|    | tgttttggca  | ccaaaatcaa  | cgggactttc  | caaaatgtcg  | taacaactcc  | gccccattga  | 540  |
|    | cgcaaattggg | cggtaggcgt  | gtacgggtggg | aggtctatat  | aagcagagct  | ctctggctaa  | 600  |
|    | ctagagaacc  | cactgcttac  | tggcttatcg  | aaattaatac  | gactcactat  | agggagaccc  | 660  |
|    | aagcttgacc  | tgcagcaagc  | ggcgcgcact  | ctactagagg  | atctttgtga  | aggaacctta  | 720  |
| 10 | cttctgtggt  | gtgacataat  | tggacaaact  | acctacagag  | atttaaagct  | ctaaggtaaa  | 780  |
|    | tttataaagt  | tttaagtgtat | aatgtgttaa  | actactgatt  | ctaattgttt  | gtgtatttta  | 840  |
|    | gattccaacc  | tatggaactg  | atgaatggga  | gcagtggtgg  | aatgccttta  | atgaggaaaa  | 900  |
|    | cctgtttttgc | tcagaagaaa  | tgccatctag  | tgatgatgag  | gctactgctg  | actctcaaca  | 960  |
|    | ttctactcct  | ccaaaaaaga  | agagaaaggt  | agaagacccc  | aaggactttc  | cttcagaatt  | 1020 |
| 15 | gctaagtttt  | ttgagtcattg | ctgtgttttag | taatagaact  | cttgcttgct  | ttgctatttta | 1080 |
|    | caccacaaag  | gaaaaagctg  | cactgctata  | caagaaaaatt | atggaaaaat  | attctgtaac  | 1140 |
|    | ctttataaagt | aggcataaca  | gttataatca  | taacatactg  | ttttttctta  | ctccacacag  | 1200 |
|    | gcatagagtg  | tctgctatta  | ataactatgc  | tcaaaaaattg | tgtaccttta  | gctttttaat  | 1260 |
| 20 | ttgtaaagggt | gttaataagg  | aatattttgat | gtatagtgcc  | ttgactagag  | atcataatca  | 1320 |
|    | gccataccac  | atgtgttagag | gttttacttg  | ctttaaaaaa  | cctccacac   | ctccccctga  | 1380 |
|    | acctgaaaca  | taaaatgaat  | gcaattgttg  | ttgttaactt  | gtttattgca  | gcttataatg  | 1440 |
|    | gttacaaata  | aagcaatagc  | atcacaaatt  | tcacaaataa  | agcatttttt  | tcactgcatt  | 1500 |
|    | ctagtgtgtg  | tttgtccaaa  | ctcatcaatg  | tatcttatca  | tgtctggatc  | cccggtgggt  | 1560 |
| 25 | accagcttcg  | tagctagaac  | atcatgttct  | gggatatcag  | cttcgtagct  | agaacatcat  | 1620 |
|    | gttctggtac  | ccccctctag  | agcgaattaa  | ttcactggcc  | gtcgttttac  | aacgtcgtga  | 1680 |
|    | ctgggaaaac  | cctggcggtta | cccaacttaa  | tcgccttgca  | gcacatcccc  | ctttcgccag  | 1740 |
|    | ctggcgtaat  | agcgaagagg  | ccgcacccga  | tcgccttcc   | caacagttgc  | gcagcctgaa  | 1800 |
|    | tggcgaaatg  | cgcctgatgc  | ggtattttct  | ccttaacgat  | ctgtgcggta  | tttcacaccg  | 1860 |
|    | catatggtgc  | actctcagta  | caatctgtct  | tgatgccgca  | tagttaagcc  | agccccgaca  | 1920 |
| 30 | cccgcacaaca | cccgtctgacg | cgcctgacg   | ggcttctctg  | ctcccggcat  | ccgcttacag  | 1980 |
|    | acaagctgtg  | accgtctccg  | ggagctgcat  | gtgtcagagg  | ttttcacccg  | catcacgaa   | 2040 |
|    | acgcgcgaga  | cgaaaggcg   | gggtaccaga  | acatgatgtt  | ctagctacga  | agctgatata  | 2100 |
|    | ccagaacatg  | atgttctagc  | tacgaagctg  | gtacccccgc  | ctcgtgatac  | gcctattttt  | 2160 |
|    | atagggttaat | gtcatgataa  | taatggtttc  | ttagacgtca  | gggtggcactt | ttcggggaaa  | 2220 |
| 35 | tgtgcgcgga  | accocctattt | gtttattttt  | ctaaatacat  | tcaaataatgt | atccgctcat  | 2280 |
|    | gagacaataa  | ccctgataaa  | tgcttcaata  | atattgaaaa  | aggaagagta  | tgagatttca  | 2340 |
|    | acatttccgt  | gtcgccttta  | ttcccttttt  | tgccgcat    | tgccctcctg  | ttttgtctca  | 2400 |
|    | cccagaaaacg | ctggtgaaag  | taaaagatgc  | tgaagatcag  | ttgggtgcac  | gagtgggtta  | 2460 |
| 40 | catcgaactg  | gatctcaaca  | gcggtaagat  | ccttgagagt  | tttcgccccg  | aagaacgttt  | 2520 |
|    | tccaattgat  | agcactttta  | aagttctgct  | atgtggcgcg  | gtattatccc  | gtattgacgc  | 2580 |
|    | cgggcaagag  | caactcggtc  | gccgcataca  | ctattctcag  | aatgacttgg  | ttgagtactc  | 2640 |
|    | accagtcaca  | gaaaagcatc  | ttacggatgg  | catgacagta  | agagaattat  | gcagtgtctg  | 2700 |
|    | cataaccatg  | agtataaca   | ctgcggccaa  | cttacttctg  | acaacgatcg  | gaggaccgaa  | 2760 |
| 45 | ggagctaacc  | gcttttttgc  | acaacatggg  | ggatcatgta  | actcgccttg  | atcgttggga  | 2820 |
|    | accggagctg  | aatgaagcca  | taccaaaccga | cgagcgtgac  | accacgatgc  | ctgtagcaat  | 2880 |
|    | ggcaacaacg  | ttgcgcaaac  | tattaactgg  | cgaactactt  | actctagctt  | cccggcaaca  | 2940 |
|    | attaatagac  | tggatggagg  | cggataaaagt | tgcaggacca  | cttctgcgct  | cggcccttcc  | 3000 |
|    | ggctggctgg  | tttattgtctg | ataaatctgg  | agccgggtgag | cgtgggtctc  | gcggtatcat  | 3060 |
| 50 | tgcagcactg  | gggcccagatg | gtaagccctc  | ccgtatcgta  | gttatctaca  | cgacggggag  | 3120 |
|    | tcaggcaact  | atggatgaac  | gaaatagaca  | gatcgctgag  | ataggtgcct  | cactgattaa  | 3180 |
|    | gcatttgtaa  | ctgtcagacc  | aagtttactc  | atatatactt  | tagattgatt  | taaaacttca  | 3240 |
|    | tttttaattt  | aaaaggatct  | aggtgaagat  | cctttttgat  | aatctcatga  | ccaaaatccc  | 3300 |
|    | ttaacgtgag  | ttttcgttcc  | actgagcgtc  | agaccccgta  | gaaaagatca  | aaggatcttc  | 3360 |
| 55 | ttgagatcct  | ttttttctgc  | gcgtaatctg  | ctgcttgcaa  | acaaaaaac   | caccgtacc   | 3420 |
|    | agcgggtggtt | tgtttgccgg  | atcaagagct  | accaactctt  | tttcggaagg  | taactggctt  | 3480 |
|    | cagcagagcg  | cagataccaa  | atactgtcct  | tctagtgtag  | ccgtagttag  | gccaccactt  | 3540 |
|    | caagaactct  | gtagcacccg  | ctacatacct  | cgctctgcta  | atcctgttac  | cagtggctgc  | 3600 |
|    | tgccagtggc  | gataagtcgt  | gtcttaaccg  | gttgactca   | agacgatagt  | taccggataa  | 3660 |
|    | ggcgagcgg   | tcgggctgaa  | cggggggttc  | gtgcacacag  | cccagcttgg  | agcgaacgag  | 3720 |
| 60 | ctacaccgaa  | ctgagatacc  | tacagcgtga  | gctatgagaa  | agcgccacgc  | ttcccgaagg  | 3780 |
|    | gagaaaggcg  | gacaggtatc  | cggtaagcgg  | cagggtcgga  | acaggagagc  | gcacgagga   | 3840 |
|    | gcttccaggg  | ggaaacgcct  | ggtatcttta  | tagtcctgtc  | gggtttcgcc  | acctctgact  | 3900 |

15

5      tgagcgtcga    tttttgtgat    gctcgtcagg    ggggcgggagc    ctatggaaaa    acgccagcaa    3960  
       cgcggccttt    ttacgggttcc    tggcctttttg    ctggcctttt    gctcacatgt    tctttcctgc    4020  
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 10      acgcaaaccg    cctctccccg    cgcgttggcc    gattcattaa    tgcagctggc    acgacaggtt    4200  
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 15      agagctctag    agctctagag    agcttgcatg    ccgggggtacc    agcttcgtag    ctagaacatc    4440  
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&lt;210&gt; 17

&lt;211&gt; 4644

15 &lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

20 &lt;223&gt; Description of Artificial Sequence: vector pTGF95

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       cgcccaacga    cccccgccc    ttgacgtcaa    taatgacgta    tgttcccata    gtaacgccc    180  
       tagggacttt    ccattgacgt    caatgggtgg    agtattttacg    gtaaactgcc    cacttggcag    240  
       tacatcaagt    gtatcatatg    ccaagtacgc    ccctatttga    cgtaaatgac    ggtaaatggc    300  
       cgcgctggca    ttatgccag    tacatgacct    tatgggactt    tcctacttgg    cagtacatct    360  
       acgtattagt    catcgctatt    accatgggtga    tgcggttttg    gcagtacatc    aatgggcgtg    420  
 30      gatagcgggt    tgactcacgg    ggatttccaa    gtctccaccc    cattgacgtc    aatgggagtt    480  
       tggttttgca    ccaaaatcaa    cgggactttc    caaaatgtcg    taacaactcc    gccccattga    540  
       cgcaaatggg    cggtaggcgt    gtacgggtggg    aggtctatat    aagcagagct    ctctggctaa    600  
       ctagagaacc    cactgcttac    tggttatcgt    aaattaatac    gactcactat    agggagaccc    660  
       aagcttgacc    tcgagcaagc    ggccgcgact    ctactagagg    atctttgtga    aggaacctta    720  
       cttctgtggt    gtgacataat    tggacaacct    acctacagag    atttaaagct    ctaaggtaaa    780  
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       gattccaacc    tatggaactg    atgaatggga    gcagtgggtg    aatgccttta    atgaggaaaa    900  
       cctgttttgc    tcagaagaaa    tgccatctag    tgatgatgag    gctactgctg    actctcaaca    960  
       ttctactcct    ccaaaaaaga    agagaaagggt    agaagacccc    aaggactttc    cttcagaatt    1020  
 40      gctaagtttt    ttgagtcatg    ctgtgtttag    taatagaact    ctgtcttgc    ttgtctattt    1080  
       caccacaaag    gaaaaagctg    cactgtctata    caagaaaatt    atggaaaaat    atctctgtaac    1140  
       ctttataagt    aggcataaca    gttataatca    taacatactg    ttttttctta    ctccacacag    1200  
       gcatagagtg    tctgctatta    ataactatgc    tcaaaaattg    tgtaccttta    gctttttaat    1260  
       ttgtaaaggg    gttaataagg    aatatttgat    gtatagtgcc    ttgactagag    atcataatca    1320  
       gccataccac    attttagtag    gttttacttg    ctttaaaaaa    cctcccaaac    ctccccctga    1380  
 45      acctgaaaca    taaaatgaat    gcaattgttg    ttgttaactt    gtttatttga    gcttataatg    1440  
       gttacaaata    aagcaatagc    atcacaaatt    tcacaaataa    agcatttttt    tcaactgcatt    1500  
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# INTERNATIONAL SEARCH REPORT

In. ational Application No

PCT/EP 00/01368

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N15/57 C12N15/67 C12N15/85 C12N9/64  
C07K14/72 C12Q1/68 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|----------|--|-----------------------|
| X        | WO 94 28150 A (UNIV MCGILL)<br>8 December 1994 (1994-12-08)  | 1,2,6,7,<br>11,29,30  |
| Y        | page 5, line 1 - line 11<br>page 6, line 34 - page 7, line 10<br>page 6, line 24 - line 28<br>page 10, line 20 - line 25<br>page 14, line 14 - line 19<br>claims 1-11<br>---<br>-/-- | 3-5,8,9               |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

6 June 2000

Date of mailing of the international search report

26/06/2000

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# INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/EP 00/01368

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |  |                       |
|--|--|-----------------------|
| Category   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
| X  | V. BOONYARATANAKORNKIT ET AL.:<br>"High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mamalian cells"<br>MOL. CELL. BIOL.,<br>vol. 18, no. 8, August 1998 (1998-08),<br>pages 4471-4487, XP002139580<br>ASM WASHINGTON, DC,US<br>cited in the application<br>the whole document | 1,2,7                 |
| X  | WO 94 17182 A (RES INST OF THE PALO ALTO MEDI ;LEAVITT JOHN C (US))<br>4 August 1994 (1994-08-04)<br>page 16, line 30 - line 36<br>page 17, line 1 - line 3; claims 1-16   | 1,2,6,7,<br>11,29,30  |
| X  | WO 93 20218 A (CONNAUGHT LAB ;FILMUS JORGE (CA); KLEIN MICHEL (CA))<br>14 October 1993 (1993-10-14)<br>the whole document  | 1,2,6,11              |
| Y  | WO 94 29471 A (GENETIC THERAPY INC)<br>22 December 1994 (1994-12-22)<br>the whole document   | 3-5,8,9               |
| A  | WO 93 23431 A (BAYLOR COLLEGE MEDICINE)<br>25 November 1993 (1993-11-25)<br>cited in the application<br>the whole document   |                       |
| A  | BEATO M ET AL: "Transcriptional regulation by steroid hormones"<br>STEROIDS: STRUCTURE, FUNCTION, AND REGULATION,US,ELSEVIER SCIENCE PUBLISHERS, NEW YORK, NY,<br>vol. 61, no. 4, 1 April 1996 (1996-04-01),<br>pages 240-251, XP004026583<br>ISSN: 0039-128X<br>the whole document  |                       |
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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01368

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| Category | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
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| A        | CROSSLEY M. ET AL: "Recovery from hemophilia B Leyden: An androgen-responsive element in the factor IX promoter." SCIENCE, (1992) 257/5068 (377-379). , XP002139582<br>the whole document   |                       |

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Information on patent family members

International Application No

PCT/EP 00/01368

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)  | Publication<br>date  |
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